

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
13 July 2006 (13.07.2006)

PCT

(10) International Publication Number  
**WO 2006/073493 A2**

(51) International Patent Classification: Not classified

(21) International Application Number:  
PCT/US2005/027044

(22) International Filing Date: 28 July 2005 (28.07.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
10/903,191 30 July 2004 (30.07.2004) US

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(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AL, AM,  
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,  
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,  
KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,  
MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ,  
OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL,  
SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC,  
VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): ARIPO (BW, GH,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,  
RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,  
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— without international search report and to be republished  
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: ANTIBODY VACCINE CONJUGATES AND USES THEREFOR

(57) Abstract: The present invention provides novel antibody vaccine conjugates and methods of using the same to induce a cy-  
totoxic T cell (CTL) response. In a particular, embodiment, the vaccine conjugate includes a human chorionic gonadotropin beta  
subunit (hCG) antigen linked to an anti-mannose receptor (MR) antibody.



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## ANTIBODY VACCINE CONJUGATES AND USES THEREFOR

### Related Applications

- This application claims priority to U.S. Provisional Patent Application  
5 No. 10/903,191, filed July 30, 2004. The entire contents of the aforementioned application is hereby incorporated herein by reference.

### Background of the Invention

- The immune response is initiated at the level of professional antigen  
10 presenting cells (APC), which include dendritic cells (DC) and macrophages (Mg), that reside in tissues throughout the body. DCs express high levels of cell surface molecules and complementary receptors that interact with T lymphocytes and, therefore, induce potent immune responses. DCs also secrete cytokines, chemokines and proteases which initiate immune responses and culminate in the amplification of both cellular and  
15 humoral immunity.

- DCs express on their surface major histocompatibility complex (MHC) molecules that bind fragments of antigens. T cells which express T cell receptors (TCR) that recognize such antigen-MHC complexes become activated and initiate the immune cascade. In general, there are two types of MHC molecules, MHC class I and MHC  
20 class II molecules. MHC class I molecules present antigen to specific CD8<sup>+</sup> T cells and MHC class II molecules present antigen to specific CD4<sup>+</sup> T cells.

- For effective treatment of many diseases, particularly cancers, vaccines must elicit a potent cytotoxic T lymphocyte (CTL) response, also referred to as a cytotoxic T cell response. Cytotoxic T cells predominantly include CD8<sup>+</sup> T cells which  
25 recognize antigen in the context of MHC class I. The processing of antigens in the context of MHC class I molecules differs significantly from that of MHC class II molecules. Antigens delivered exogenously to APCs are processed primarily for association with MHC class II molecules. In contrast, due to the intracellular location of MHC class I molecules, antigens delivered endogenously to APCs are processed  
30 primarily for association with MHC class I molecules. This is not only true for APCs, as all nucleated cells express MHC class I molecules, and are continuously displaying on their surface endogenously produced antigens in association with MHC class I molecules.

- For this reason, cells infected with virus or tumor cells expressing unique  
35 proteins can be targeted by CTLs when viral or tumor antigens are displayed as a peptide bound to MHC class I molecules. However, DCs, under specific conditions, have the unique capacity also to allow exogenous antigens access to internal compartments for

binding to MHC class I molecules, so that they are presented to T cells via both MHC class I and class II pathways. This process is called cross-priming or cross-presentation.

Accordingly, while antibody-mediated responses have demonstrated impressive protective or therapeutic efficacy for specific diseases when directed against particular secreted or cell surface antigens, the most effective immunotherapy for many diseases appears to require T cell-mediated immune responses, particularly CTL responses. Since effective CTL responses are not limited to extracellular antigens, there exist possibilities for developing antigen-based therapeutic vaccines that are not effective antibody targets. Therefore, new methods for generating CTLs in response to disease-associated antigens have been of great interest, as these cells are thought to be critical for the efficacy of many vaccines in general, and essential to most therapeutic cancer vaccines.

One vaccine approach which has been tested to date employs immunizing with antigenic peptides. This method of immunization bypasses the need for antigen uptake and processing and relies on the ability of the peptide to bind directly to MHC class I molecules already expressed on the surface of the APC. Although this method has clearly shown evidence of CTL induction in patients, the method has several limitations. The antigenic peptide must be pre-established, different peptides are required for individuals with different MHC haplotypes, and peptides are short-lived *in vivo*.

Another approach which has been tested employs antibody-antigen complexes. Paul *et al.* (62) showed that antibodies specific for a given antigen could enhance humoral immune responses against the antigen in mice, presumably by delivering the immune complexes to Fc receptors for IgG (FcγR) expressed on APCs. Wernersson and colleagues (63) studied the role of individual FcγRs in the enhancement of immune responses using immune complexes *in vivo*. Their studies demonstrated that FcγRI is sufficient to mediate enhanced immune responses. However, such immune complexes do not target APCs specifically, as they also bind to Fc receptors on many cells that are not involved in antigen presentation, thereby, decreasing the efficiency of antigen delivery.

Subsequent studies have used antibodies to selectively target antigens to a variety of receptors on APCs, and have demonstrated that such selective delivery is capable of inducing humoral responses (66,67). In addition, it has been shown that immune complexes bound to FcR on DCs are processed and presented in context of MHC class I (64,65). Moreover, many such FcR-targeting approaches are limited because FcR are expressed on many non-APC such as platelets and neutrophils. Ideally, a vaccine that targets APC specifically and is capable of inducing an effective MHC

class I-restricted CTL response, as well as an effective MHC class II – restricted TH response could offer improved efficacy in treating certain diseases.

Similarly, mannosylated antigens have been shown to induce humoral immune responses and T cell-mediated immune responses, such as CTL responses.

5 However, mannosylated antigens do not target APC specifically due to the significant abundance of other mannose binding proteins. Furthermore, mannosylated proteins are internalized by immature DCs through macropinocytic mechanisms. Therefore, the mechanisms and nature of immune responses generated by mannosylation of antigens differs greatly from that generated by specific targeting of antigens to mannose receptors  
10 using antibodies.

Since current methods do not efficiently and specifically target APCs, many therapeutic vaccines require the purification of DC from patients, which are reinfused after exposure to the antigen.

Accordingly, the need exists for improved vaccines capable of efficiently  
15 targeting APCs and generating antigen-specific T cell-mediated immune responses, including antigen-specific CTL responses, required for effective treatment of many diseases.

### Summary of the Invention

20 The present invention provides antibody-based vaccines and methods for generating antigen-specific T cell-mediated immune responses required for effective treatment of many diseases. In particular, a potent antigen-specific cytotoxic T lymphocyte (CTL) response is induced by targeting one or more protein antigens to antigen presenting cells (APCs), using antibodies which bind to particular receptors  
25 expressed on APCs. Preferred receptors include C-lectins, particularly the human mannose receptor, which are expressed on both dendritic cells (DCs) and macrophages. As demonstrated by way of the present invention, targeting the mannose receptor using antibody-antigen conjugates results in processing of the antigen through both MHC class I and class II pathways. Thus, antigen-specific CTLs (*e.g.*, CD8<sup>+</sup> T cells) are induced, as  
30 well as other important effector T cells, including helper T cells (*e.g.*, CD4<sup>+</sup> T cells).

Accordingly, in one aspect, the present invention provides a method for inducing or enhancing a CTL response against an antigen by forming a conjugate of the antigen and a monoclonal antibody which binds to a human APC, *e.g.*, a monoclonal antibody which binds to the human mannose receptor expressed on human APC. The  
35 conjugate is then contacted, either *in vivo* or *ex vivo*, with APCs such that the antigen is internalized, processed and presented to T cells in a manner which induces or enhances a CTL response (*e.g.*, a response mediated by CD8<sup>+</sup> cytotoxic T cells) against the antigen. In a preferred embodiment, this serves also to induce a helper T cell response (*e.g.*, a

response mediated by CD4<sup>+</sup> helper T cells) against the antigen. Thus, the immune response is induced through both MHC class I and MHC class II pathways. The APCs can also be contacted with an adjuvant or an immunostimulatory agent, such as a cytokine, which stimulates proliferation of dendritic cells to further enhance the immune response. Such agents can be contacted separately from the molecular conjugate or linked to the molecular conjugate.

Accordingly, in a particular embodiment, the invention provides a vaccine conjugate comprising a monoclonal antibody which binds to a human APC linked to an antigen and to an immunostimulatory agent. The immunostimulatory agent can be linked to the conjugate either covalently or non-covalently. Alternatively, the immunostimulatory agent can be genetically fused to the conjugate (*e.g.*, expressed together with the conjugate as a single fusion protein). A variety of suitable immunostimulatory agents can be employed including, but not limited to: CD40 ligand; cytokines, such as IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$  and IL-2; colony-stimulating factors, such as G-CSF (granulocyte colony-stimulating factor) and GM-CSF (granulocyte-macrophage colony-stimulating factor); an anti-CTLA-4 antibody; toll receptor agonists (*e.g.*, flagellin and MALP-2 (macrophage activating lipopeptide-2); LPS (endotoxin); R837 (3M Pharmaceuticals, St. Paul, MN); R848 (3M Pharmaceuticals, St. Paul, MN); polyI:C (inosine:cytosine polynucleotide); ssRNA; dsRNA; Bacille Calmette-Guerin (BCG); Levamisole hydrochloride; and intravenous immune globulins.

A variety of suitable antibodies can be employed in the conjugates of the present invention including, but not limited to those derived from any species (*e.g.*, human, murine, rabbit etc.) and/or those engineered and expressed recombinantly (*e.g.*, chimeric, humanized and human antibodies). Preferred antibodies include human monoclonal antibodies. Antibodies used in the invention also can include any antibody isotype, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, or IgE, although preferred antibodies are of the IgG isotype. The antibodies can be whole antibodies or antigen-binding fragments thereof including, for example, Fab, F(ab')<sub>2</sub>, Fv and single chain Fv fragments.

Preferred antibodies for use in the present invention include human monoclonal antibodies that bind to the human mannose receptor. In one embodiment, the antibody is encoded by human heavy chain and human kappa light chain nucleic acids comprising nucleotide sequences in their variable regions as set forth in SEQ ID NO:3 and SEQ ID NO:7, respectively, or a nucleotide sequence that is sufficiently homologous to SEQ ID NO:3 or SEQ ID NO:7 such that the antibody retains the ability to bind to dendritic cells.

Still other preferred human antibodies include those characterized as binding to the human mannose receptor and having a human heavy chain and human kappa light chain variable regions comprising the amino acid sequences as set forth in SEQ ID NO:4 and SEQ ID NO:8, respectively, or an amino acid sequence that is  
5 sufficiently homologous to SEQ ID NO:4 or SEQ ID NO:8 such that the antibody retains the ability to bind to dendritic cells.

Still other particular human antibodies of the invention include those which comprise a complementarity determining region (CDR) domain having a human heavy and light chain CDR1 region, a human heavy and light chain CDR2 region, and a  
10 human heavy and light chain CDR3 region, wherein

(a) the CDR1, CDR2, and CDR3 of the human heavy chain regions comprise an amino acid sequence selected from the group consisting of the amino acid sequences of the CDR1, CDR2, and CDR3 regions shown in Figure 8 (SEQ ID NOs:13, 14, or 15), and conservative sequence modifications thereof, and

15 (b) the CDR1, CDR2, and CDR3 of the human light chain regions comprise an amino acid sequence selected from the group consisting of the amino acid sequences of the CDR1, CDR2, and CDR3 regions shown in Figure 9 (SEQ ID NOs:16, 17, or 18), and conservative sequence modifications thereof.

Antibodies derived from a particular germline sequence, for example,  
20 antibodies obtained from a system using human immunoglobulin sequences, *e.g.*, by immunizing a transgenic mouse carrying human immunoglobulin genes or by screening a human immunoglobulin gene library, are also included in the present invention.

Human antibodies for use in the invention can be produced recombinantly in a host cell, such as a transfectoma (*e.g.*, a transfectoma consisting of  
25 immortalized CHO cells or lymphocytic cells) containing nucleic acids encoding the heavy and light chains of the antibody, or be obtained directly from a hybridoma which expresses the antibody (*e.g.*, which includes a B cell obtained from a transgenic nonhuman animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene that encode the antibody, fused  
30 to an immortalized cell). In a particular embodiment, the antibodies are produced by a hybridoma, or by a host cell (*e.g.*, a CHO cell) transfectoma containing human heavy chain and human light chain nucleic acids which comprise nucleotide sequences SEQ ID NOs:3 and 7, respectively, and conservative modifications thereof.

Suitable antigens for use in the present invention include any antigen, or  
35 antigenic portion thereof, against which a protective or therapeutic immune responses is desired including, for example, a variety of tumor and infectious disease antigens. Particular antigens can be selected from, among others, human chorionic gonadotropin beta subunit ( $\beta$ hCG), Gp100, prostate associated antigen (PSA), Pmel-17, colon, lung,

pancreas, breast, ovary, and germ cell derived tumor cell antigens, viral proteins, bacterial proteins, carbohydrates, and fungal proteins. In accordance with the invention, such antigens are linked to antibodies to form highly effective antibody vaccine conjugates.

5 In another aspect, the present invention provides a particular antibody vaccine conjugate that includes  $\beta$ hCG linked to an antibody which binds to the human mannose receptor. In one embodiment, the conjugate comprises a human heavy chain which is linked to  $\beta$ hCG, such as the B11- $\beta$ hCG conjugate described herein having a heavy chain comprising the amino acid sequence shown in SEQ ID NO:10. A single  
10 chain version of the B11- $\beta$ hCG conjugate is also provided, comprising the amino acid sequence shown in SEQ ID NO:12.

The present invention further provides compositions (*e.g.*, pharmaceutical compositions) containing one or more antibody vaccine conjugates of the invention.

The compositions can additionally include one or more adjuvants or other  
15 agents known to enhance immune responses and/or increase the activity of APCs.

Other features and advantages of the instant invention will be apparent from the following detailed description and claims.

### Brief Description of the Drawings

20

*Figure 1* shows a map of the molecular conjugate (SEQ ID NOs:11 and 12) encoding a fusion protein containing the single chain B11 antibody linked to  $\beta$ hCG antigen (pB11sfv- $\beta$ hCG).

25

*Figure 2* shows a map of the molecular conjugate (SEQ ID NOs:9 and 10) encoding a fusion protein containing the whole B11 antibody linked to  $\beta$ hCG antigen ( $\beta$ hCG-B11 construct).

*Figure 3* is a schematic illustration of a molecular conjugate. The antigen  
30 is genetically fused to the heavy chains of the intact antibody.

*Figure 4* is a graph based on flow cytometry studies which shows that the  $\beta$ hCG-B11 construct binds specifically to cultured human DC expressing MR.

35

*Figure 5* is a graph showing that the  $\beta$ hCG-B11 construct induces  $\beta$ hCG-specific cytotoxic T cells.

*Figure 6* is a graph showing that the  $\beta$ hCG-B11 construct induces  $\beta$ hCG-specific cytotoxic T cells.

*Figure 7* is a bar graph showing that the  $\beta$ hCG-B11 construct induces T  
5 helper response.

*Figure 8* shows the nucleotide sequence (SEQ ID NO:3) and  
corresponding amino acid sequence (SEQ ID NO:4) of the heavy chain V region of  
human monoclonal antibody B11 with CDR regions designated (SEQ ID NOs: 13, 14,  
10 and 15).

*Figure 9* shows the nucleotide sequence (SEQ ID NO:7) and  
corresponding amino acid sequence (SEQ ID NO:8) of the light (kappa) chain V region  
of human monoclonal antibody B11 with CDR regions designated (SEQ ID NOs: 16, 17,  
15 and 18).

*Figure 10* is a diagram showing the predicted T cell epitopes of the  
 $\beta$ hCG-B11 construct as analyzed using web-based predictive algorithms (BIMAS &  
SYFPEITHI). T cell epitopes were found for potential binding to HLA-A2, HLA-B7  
20 and HLA-DR molecules. Several epitopes were also predicted from the B11 segment of  
 $\beta$ hCG-B11. No T cell epitope was identified in the 37 aa long C-terminal peptide.

*Figure 11* is a graph showing CTL specific for the  $\beta$ hCG-B11 construct  
recognize the scFv form of the antigen, B11sfv- $\beta$ hCG presented by DCs.  
25

*Figure 12* shows the amino acid sequence (SEQ ID NO:4) of the heavy  
chain V region of human monoclonal antibody B11 compared to the germline sequence  
(SEQ ID NO:30), VH5-51 germline.

*Figure 13* shows the nucleotide sequence (SEQ ID NO:3) of the heavy  
chain V region of human monoclonal antibody B11 compared to the germline sequence  
(SEQ ID NO:29), VH5-51 germline.  
30

*Figure 14* shows the amino acid sequence (SEQ ID NO:8) of the light  
35 (kappa) chain V region of human monoclonal antibody B11 with CDR regions  
designated compared to the germline sequence (SEQ ID NO:32), Vk-L15 germline.



*Figure 15* shows the nucleotide sequence (SEQ ID NO:7) of the light (kappa) chain V region of human monoclonal antibody B11 with CDR regions designated compared to the germline sequence (SEQ ID NO:31), Vk-L15 germline.

5           *Figure 16* shows targeted uptake of B11- $\beta$ hCG antigen by interstitial dendritic cells and lymph nodes, as well as macrophages of surrounding tissues, in transgenic mice that express the human mannose receptor (huMR-tg mice).

10           *Figure 17* is a graph showing enhanced antigen-specific humoral response to B11- $\beta$ hCG in huMR-tg mice compared to control mice and compared to nontargeted  $\beta$ hCG.

15           *Figure 18* is a graph showing enhanced antigen-specific cellular response to B11- $\beta$ hCG in huMR-tg mice compared to control mice and compared to nontargeted  $\beta$ hCG.

#### **Detailed Description of the Invention**

The present invention is based on the discovery that important T cell-mediated immune responses can be generated by targeting antigens to antigen presenting cells (APCs) using antibodies directed against particular cellular receptors. Specifically, for effective treatment of many diseases, such as cancers and infectious diseases, vaccines must elicit a potent antigen-specific cytotoxic T lymphocyte (CTL) response, primarily mediated by CD8+ T cells which recognize antigen in the context of MHC class I. For optimal immunization, this is preferably accompanied by other important effector T cell functions, including induction of antigen-specific helper T cells, such as CD4+ T cells, which recognize antigen in the context of the MHC class II pathway. Thus, effective vaccines should induce antigen-specific CTLs, preferably in combination with other T cell-mediated immune responses, through multiple MHC pathways.

Accordingly, the present invention provides novel antibody-based vaccine conjugates and methods for inducing or enhancing antigen-specific cytotoxic T cell (CTL) responses. Therapies of the invention employ molecular conjugates comprising antibodies which bind to antigen presenting cells (APC), such as dendritic cells (DC) and macrophages, linked to an antigen. The molecular conjugates can be administered alone or with other immunostimulatory agents and/or adjuvants that further enhance the immune response against the antigen. In one embodiment, the immunostimulatory agent is co-administered with the molecular conjugate. In another embodiment, the immunostimulatory agent is administered prior to or after administration of the molecular conjugate. In yet another embodiment, the

immunostimulatory agent is linked (*e.g.*, covalently, non-covalently or recombinantly) to the molecular conjugate. For example, the immunostimulatory agent can be genetically fused or chemically linked to the molecular conjugate via, *e.g.*, the heavy and/or light chain of the antibody portion of the conjugate.

5                   Antibodies which target APCs are known in the art and include, for example, antibodies which target Class I or Class II major histocompatibility (MHC) determinants on APC (78, 79, 81, 83). Other antibodies include those which target Fc receptors on APCs (77, 79, 80, 81, 82, 83), as well as surface immunoglobulins on B cells (84).

10                   In a particular embodiment exemplified herein, the molecular conjugate includes an antibody which binds to the mannose receptor (MR) on human DCs, linked to the  $\beta$ hCG antigen. Such conjugates can be contacted with APCs either *in vivo* or *ex vivo* to generate desired CTL responses.

                  In order that the present invention may be more readily understood, 15 certain terms are first defined. Additional definitions are set forth throughout the detailed description.

                  As used herein, the term "antigen presenting cell (APC)" refers to a class of immune cells capable of internalizing and processing an antigen, so that antigenic determinants are presented on the surface of the cell as MHC-associated complexes, in a 20 manner capable of being recognized by the immune system (*e.g.*, MHC class I restricted cytotoxic T lymphocytes and/or MHC class II restricted helper T lymphocytes). The two requisite properties that allow a cell to function as an APC are the ability to process endocytosed antigens and the expression of MHC gene products. Examples of APCs include dendritic cells (DC), mononuclear phagocytes (*e.g.*, macrophages), B 25 lymphocytes, Langerhans cells of the skin and, in humans, endothelial cells.

                  The term "dendritic cell (DC)" as used herein, includes immature and mature DCs and related myeloid progenitor cells that are capable of differentiating into DCs or related antigen presenting cells (*e.g.*, monocytes and macrophages). DCs express high levels of cell surface molecules and complementary receptors that interact 30 with T lymphocytes (*e.g.*, C-type lectins, such as the mannose receptor) and, therefore, are capable of inducing potent immune responses. DCs also secrete cytokines, chemokines and proteases which initiate an immune response and culminates in the amplification of both cellular and humoral immunity. DCs also express on their surface major histocompatibility complex (MHC) molecules that bind fragments of antigens. T 35 cells which recognize these antigen-MHC complexes become activated and initiate the immune cascade. In a preferred embodiment, binding of an antibody portion of the molecular conjugate of the invention to a dendritic cell results in internalization of the conjugate by the dendritic cell.

The term "macrophage mannose receptor" or "MR" refers to a member of a family of C-type lectin receptors characterized by repeated carbohydrate-recognition domains (CRD) in the extracellular portion and a short cytoplasmic tail containing two putative clathrin targeting sequences (34,35,37). In addition, the MR contains N-terminal cysteine rich and fibronectin domains. The different domains of the mannose receptor have specific binding capacity for various ligands including lysosomal enzymes, micro-organisms, pituitary hormones, glycosaminoglycans, and sulfated blood group antigens (38-40).

"MHC molecules" include two types of molecules, MHC class I and MHC class II. MHC class I molecules present antigen to specific CD8<sup>+</sup> T cells and MHC class II molecules present antigen to specific CD4<sup>+</sup> T cells. Antigens delivered exogenously to APCs are processed primarily for association with MHC class II. In contrast, antigens delivered endogenously to APCs are processed primarily for association with MHC class I. However, under specific conditions, DCs have the unique capacity to allow exogenous antigens access to internal compartments for binding to MHC class I molecules, in addition to MHC class II molecules. This process is called "cross-priming" or "cross-presentation."

As used herein, the term "immunostimulatory agent" refers to compounds capable of stimulating APCs, such as DCs and macrophages. For example, suitable immunostimulatory agents for use in the present invention are capable of stimulating APCs so that the maturation process of the APCs is accelerated, the proliferation of APCs is increased, and/or the recruitment or release of co-stimulatory molecules (*e.g.*, CD80, CD86, ICAM-1, MHC molecules and CCR7) and pro-inflammatory cytokines (*e.g.*, IL-1 $\beta$ , IL-6, IL-12, IL-15, and IFN- $\gamma$ ) is upregulated. Suitable immunostimulatory agents are also capable of increasing T cell proliferation. Such immunostimulatory agents include, but are not be limited to, CD40 ligand; cytokines, such as IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$  and IL-2; colony-stimulating factors, such as G-CSF (granulocyte colony-stimulating factor) and GM-CSF (granulocyte-macrophage colony-stimulating factor); an anti-CTLA-4 antibody; toll receptor agonists (*e.g.*, flagellin and MALP-2 (macrophage activating lipopeptide-2); LPS (endotoxin); R837 (3M Pharmaceuticals, St. Paul, MN); R848 (3M Pharmaceuticals, St. Paul, MN); polyI:C (inosine:cytosine polynucleotide); ssRNA; dsRNA; Bacille Calmette-Guerin (BCG); Levamisole hydrochloride; and intravenous immune globulins.

As used herein, the term "linked" refers to the association of two or more molecules. The linkage can be covalent or non-covalent. The linkage also can be genetic (*i.e.*, recombinantly fused). Such linkages can be achieved using a wide variety of art recognized techniques, such as chemical conjugation and recombinant protein production.

As used herein, the term antigen "cross-presentation" refers to presentation of exogenous protein antigens to T cells via MHC class I and class II molecules on APCs.

As used herein, the term "T cell-mediated response" refers to any response mediated by T cells, including effector T cells (*e.g.*, CD8<sup>+</sup> cells) and helper T cells (*e.g.*, CD4<sup>+</sup> cells). T cell mediated responses include, for example, T cell cytotoxicity and proliferation.

As used herein, the term "cytotoxic T lymphocyte (CTL) response" refers to an immune response induced by cytotoxic T cells. CTL responses are mediated primarily by CD8<sup>+</sup> T cells.

As used herein, the term "antibody" includes whole antibodies or antigen-binding fragments thereof including, for example, Fab, F(ab')<sub>2</sub>, Fv and single chain Fv fragments. Suitable antibodies include any form of antibody, *e.g.*, murine, human, chimeric, or humanized and any type antibody isotype, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, or IgE isotypes. As used herein, "isotype" refers to the antibody class that is encoded by heavy chain constant region genes.

Whole antibodies contain at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or V<sub>H</sub>) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or V<sub>L</sub>) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The V<sub>H</sub> and V<sub>L</sub> regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions (CDR)", interspersed with regions that are more conserved, termed framework regions (FR). Each V<sub>H</sub> and V<sub>L</sub> is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

Preferred antibodies of the invention include human antibodies, *e.g.*, a human antibody having an IgG1 (*e.g.*, IgG1k) heavy chain and a kappa light chain. Other preferred antibodies of the invention bind human DCs, such as antibodies which bind a C-type lectin receptor on a human DC, *e.g.*, the MR on human DCs. In a particular embodiment, the antibody is a human monoclonal antibody that binds to the human macrophage mannose receptor (also referred to herein as "human B11 antigen")

having an approximate molecular weight of 180 kD as measured by SDS-PAGE.

Protocols for generating such antibodies are described in WO 01/085798, the contents of which are incorporated herein by reference. Particular human antibodies include those which comprise heavy and light chain variable regions amino acid sequences as shown  
5 in SEQ ID NOs: 2 and 6, respectively, or an amino acid sequence that is sufficiently homologous to SEQ ID NO:2 or SEQ ID NO:6 such that the antibody retains the ability to bind to dendritic cells

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the  
10 ability to specifically bind to an antigen (*e.g.*, an antigen on a dendritic cell). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')<sub>2</sub> fragment, a  
15 bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore,  
20 although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-  
25 5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The term "human antibody," as used herein, is intended to include  
30 antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended  
35 to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The terms "monoclonal antibody" or "monoclonal antibody composition," as used herein, refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable and constant regions derived from human germline immunoglobulin sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene, fused to an immortalized cell.

The term "recombinant human antibody," as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody, *e.g.*, from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the  $V_H$  and  $V_L$  regions of the recombinant antibodies are sequences that, while derived from and related to human germline  $V_H$  and  $V_L$  sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

As used herein, "specific binding" refers to antibody binding to a predetermined antigen. Typically, the antibody binds with a dissociation constant ( $K_D$ ) of  $10^{-7}$  M or less, and binds to the predetermined antigen with a  $K_D$  that is at least two-fold less than its  $K_D$  for binding to a non-specific antigen (*e.g.*, BSA, casein) other than the predetermined antigen or a closely-related antigen. The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen."

As used herein, the term "high affinity" for an IgG antibody refers to an antibody having a  $K_D$  of  $10^{-8}$  M or less, more preferably  $10^{-9}$  M or less and even more preferably  $10^{-10}$  M or less. However, "high affinity" binding can vary for other antibody

isotypes. For example, "high affinity" binding for an IgM isotype refers to an antibody having a  $K_D$  of  $10^{-7}$  M or less, more preferably  $10^{-8}$  M or less.

The term " $K_{\text{assoc}}$ " or " $K_a$ ", as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term " $K_{\text{dis}}$ " or " $K_d$ ", as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term " $K_D$ ", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of  $K_d$  to  $K_a$  (*i.e.*,  $K_d/K_a$ ) and is expressed as a molar concentration (M).

As used herein, the term " $\beta$ hCG" refers to the beta subunit of human chorionic gonadotropin and includes the whole antigen, antigenic fragments thereof, allelic variants thereof, and any polymorphisms, derived from the  $\beta$ hCG sequence (SEQ ID NO:20).  $\beta$ hCG is a hormone necessary for the establishment of a successful pregnancy. Aside from pregnancy, the expression of this antigen is primarily restricted to germ cell tumors, as well as a significant number of adenocarcinomas.

The term "nucleic acid molecule", as used herein, is intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule," is used herein in reference to nucleic acids encoding the molecular conjugates of the invention or portions thereof, *e.g.*, SEQ ID NOs:9 and 11 or portions thereof, such as the antigen or antibody portions (*i.e.*, the  $V_H$ ,  $V_L$ , or CDRs). Isolated nucleic acid molecules refer to a nucleic acid molecule in which the nucleotide sequences encoding the molecular conjugates are free of other contaminating nucleotide sequences, *e.g.*, a nucleotide sequence which does not encode any part of the molecular conjugate.

As disclosed and claimed herein, the sequences set forth in SEQ ID NOs: 1-28 can include "conservative sequence modifications," *i.e.*, nucleotide and amino acid sequence modifications which do not significantly affect or alter the functional characteristics of the molecular conjugate, *e.g.*, the binding properties of the antibody portion of the construct or the immunogenic properties of the antigen portion, encoded by the nucleotide sequence or containing the amino acid sequence. Such conservative sequence modifications include nucleotide and amino acid substitutions, additions and deletions. Modifications can be introduced into SEQ ID NOs: 1-28 by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions include ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*,

glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted  
5 nonessential amino acid residue in a human anti-DCs antibody is preferably replaced with another amino acid residue from the same side chain family.

Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a molecular conjugate coding sequence, such as by saturation mutagenesis, and the resulting modified molecular conjugates can be screened  
10 for appropriate functional activity.

Accordingly, molecular conjugates encoded by the nucleotide sequences disclosed herein and/or containing the amino acid sequences disclosed herein (*i.e.*, SEQ ID NOs: 1-28) include substantially similar conjugates encoded by or containing similar sequences which have been conservatively modified. In particular, discussion as to how  
15 substantially similar antibodies can be generated for use in the molecular conjugates based on the partial (*i.e.*, heavy and light chain variable regions) sequences (SEQ ID NOs: 3, 4, 7, and 8) is provided below.

For nucleic acids, the term "substantial homology" indicates that two nucleic acids, or designated sequences thereof, when optimally aligned and compared,  
20 are identical, with appropriate nucleotide insertions or deletions, in at least about 80% of the nucleotides, usually at least about 90% to 95%, and more preferably at least about 98% to 99.5% of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions, to the complement of the strand.

The percent identity between two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between  
30 two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60,  
35 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue



table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a  
5 Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the  
10 NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences  
15 homologous to the protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

20 The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel  
25 electrophoresis and others well known in the art. See, F. Ausubel, *et al.*, ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987).

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is  
30 operably linked to a coding sequence if it affects the transcription of the sequence. With respect to transcription regulatory sequences, operably linked means that the DNA sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. For switch sequences, operably linked indicates that the sequences are capable of effecting switch recombination.

35 The term "vector," as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral

vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. Recombinant host cells include, for example, CHO cells and lymphocytic cells.

As used herein, the term "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as nonhuman primates, sheep, dog, cow, chickens, amphibians, reptiles, etc.

Various aspects of the invention are described in further detail in the following subsections.

### 30 I. Antigens

Suitable antigens for use in the present invention include, for example, infectious disease antigens and tumor antigens, against which protective or therapeutic immune responses are desired, *e.g.*, antigens expressed by a tumor cell or a pathogenic organism or infectious disease antigens. For example, suitable antigens include tumor-associated antigens for the prevention or treatment of cancers. Examples of tumor-associated antigens include, but are not limited to,  $\beta$ hCG, gp100 or Pmel17, HER2/neu, CEA, gp100, MART1, TRP-2, melan-A, NY-ESO-1, MN (gp250), idiotype, MAGE-1, MAGE-3, Tyrosinase, Telomerase, MUC-1 antigens, and germ cell derived tumor

antigens. Tumor associated antigens also include the blood group antigens, for example, Le<sup>a</sup>, Le<sup>b</sup>, LeX, LeY, H-2, B-1, B-2 antigens. Alternatively, more than one antigen can be included within the antigen-antibody constructs of the invention. For example, a MAGE antigen can be combined with other antigens such as melanin A, tyrosinase, and gp100 along with adjuvants such as GM-CSF or IL-12, and linked to an anti-APC antibody.

Other suitable antigens include viral antigens for the prevention or treatment of viral diseases. Examples of viral antigens include, but are not limited to, HIV-1 gag, HIV-1 env, HIV-1 nef, HBV core, FAS, HSV-1, HSV-2, p17, ORF2 and ORF3 antigens. Examples of bacterial antigens include, but are not limited to, *Toxoplasma gondii* or *Treponema pallidum*. The antibody-bacterial antigen conjugates of the invention can be in the treatment or prevention of various bacterial diseases such as Anthrax, Botulism, Tetanus, Chlamydia, Cholera, Diptheria, Lyme Disease, Syphilis and Tuberculosis.

In a particular embodiment exemplified herein, the present invention employs an antigen comprising  $\beta$ hCG. This includes the entire  $\beta$ hCG sequence (SEQ ID NO:20) or any immunogenic (*e.g.*, T cell epitope containing) portion of the sequence. As described below, such immunogenic portions can be identified using techniques known in the art for mapping T cell epitopes, including algorithms and known T cell epitope mapping techniques. Examples of particular immunogenic peptides from  $\beta$ hCG include those comprising SEQ ID NOs:21, 22, 23, 24, 25, 26, 27, or 28, and conservative modifications thereof. Additional immunogenic peptides from  $\beta$ hCG, and methods for identifying such peptides, are described in U.S. Patent Nos. US 6,096,318 and 6,146,633, the contents of which are incorporated by reference herein.

Antigenic peptides of proteins (*i.e.*, those containing T cell epitopes) can be identified in a variety of manners well known in the art. For example, T cell epitopes can be predicted by analyzing the sequence of the protein using web-based predictive algorithms (BIMAS & SYFPEITHI) to generate potential MHC class I and II- binding peptides that match an internal database of 10,000 well characterized MHC binding peptides previously defined by CTLs. High scoring peptides can be ranked and selected as "interesting" on the basis of high affinity to a given MHC molecule. As shown in Figure 10 and using the sequence of the  $\beta$ hCG-B11 conjugate (SEQ ID NO:10), both algorithms were used to identify antigenic peptides from the  $\beta$ hCG portion (mustard) from which synthetic versions could be made and tested for their capacity to elicit T cell responses *in vitro*. Thus, T cell epitopes were found for potential binding to HLA-A2, HLA-B7 and HLA-DR molecules. Several epitopes were also predicted from the antibody (B11) segment of the  $\beta$ hCG-B11 conjugate (results not shown). Further, no T cell epitope was identified in the 37 amino acid long C-terminal peptide (CTP).

Another method for identifying antigenic peptides containing T cell epitopes is by dividing the protein into non-overlapping peptides of desired length or overlapping peptides of desired lengths which can be produced recombinantly, synthetically, or in certain limited situations, by chemical cleavage of the protein and  
5 tested for immunogenic properties, *e.g.*, eliciting a T cell response (*i.e.*, proliferation or lymphokine secretion).

In order to determine precise T cell epitopes of the protein by, for example, fine mapping techniques, a peptide having T cell stimulating activity and thus comprising at least one T cell epitope, as determined by T cell biology techniques, can  
10 be modified by addition or deletion of amino acid residues at either the amino or carboxy terminus of the peptide and tested to determine a change in T cell reactivity to the modified peptide. If two or more peptides which share an area of overlap in the native protein sequence are found to have human T cell stimulating activity, as determined by T cell biology techniques, additional peptides can be produced  
15 comprising all or a portion of such peptides and these additional peptides can be tested by a similar procedure. Following this technique, peptides are selected and produced recombinantly or synthetically. Peptides are selected based on various factors, including the strength of the T cell response to the peptide (*e.g.*, stimulation index). The physical and chemical properties of these selected peptides (*e.g.*, solubility, stability) can then be  
20 examined to determine whether the peptides are suitable for use in therapeutic compositions or whether the peptides require modification.

## II. Antibody Vaccine Conjugates

The present invention provides a variety of therapeutic vaccine  
25 conjugates which include an antigen, such as a tumor or viral antigen, linked to an antibody that binds to an APC, *e.g.*, *via* the mannose receptor (MR). This allows for targeting of the antigen to APCs (*e.g.*, dendritic cells) to enhance processing, presentation and, ultimately, an immune response against the antigen(s), *e.g.*, a CTL response.

30 In addition, the vaccine conjugate can include one or more immunostimulatory agents that also enhance the immune response against the antigen. Antibody-antigen vaccine conjugates of the invention can be made genetically or chemically. In either case, the antibody portion of the conjugate may consist of the whole antibody or a portion of the antibody, such as the Fab fragment or single-chain  
35 Fv. In addition, more than one antigen and/or immunostimulatory agent can be included in the conjugate.

In one embodiment, the vaccine conjugate comprises a human antibody heavy chain that binds to human APCs and a human antibody light chain that binds to human APCs, wherein either or both chains are linked to the antigen and to an immunostimulatory agent. In another embodiment, the antigen and the  
 5 immunostimulatory agent are separately linked to either chain. In a particular embodiment, the antigen is  $\beta$ hCG.

Genetically constructed anti-dendritic antibody-antigen conjugates (*e.g.*, those expressed as a single recombinant fusion protein) can be made by linking an antigen of choice and/or an immunostimulatory agent (in the case of protein and peptide  
 10 immunostimulatory agents) to the antibody at a variety of locations. Particular genetically produced conjugates (fusion constructs) of the invention include, for example, the  $\beta$ hCG-B11 construct, shown in Figure 2. The  $\beta$ hCG-B11 construct comprises human anti-dendritic cell antibody B11 fused to  $\beta$ hCG, a tumor-associated antigen. The nucleotide sequence encoding this construct is shown in SEQ ID NO:9.

For example, in one embodiment, the  $\beta$ hCG antigen and/or the  
 15 immunostimulatory agent can be fused to the end of the CH<sub>3</sub> domain of the human antibody heavy chain. The antigen and/or immunostimulatory agent also can be fused at the hinged region of the antibody heavy chain in Fab-fusion constructs, or in sequence with the variable light and heavy chains (V<sub>H</sub> and V<sub>L</sub>) in single chain fusion constructs  
 20 (ScFv constructs). Alternatively, the antigen and/or immunostimulatory agent can be fused to the antibody light chain instead of the antibody heavy chain. Other points of fusion among the immunostimulatory agent, the antigen and the antibody can be used provided the genetic fusion construct can elicit a CTL response. A detailed map of the intact  $\beta$ hCG-B11 construct and the single chain B11 construct (pB11sfv- $\beta$ hCG) are  
 25 shown in Tables 1 and 2, respectively. Such genetic fusion conjugates can include an antigen and an immunostimulatory agent linked to the antibody in either order (*e.g.*, an antibody-antigen-immunostimulatory conjugate or an antibody-immunostimulatory agent-antigen conjugate).

30 **Table 1:  $\beta$ hCG-B11 Feature Map**

CDS (3 total)

BUSfr-bHCG

Start: 921 End: 2153 neo

Start 3375 End: 4169 neomycin resistance gene

35 Amp

Start: 5671 End: 6531 (Complementary) Ampicillin resistance gene

Misc. Feature (5 total)

promoter

	Start: 863 End: 882 promoter signal sequence
	Start 921 End: 977 B11 VL
	Start: 978 End: 1296 B11 VH
5	Start: 1344 End: 1691 beta HCG
	Start: 1712 End: 2164
	PolyA Signal (2 total)
	poly A
	Start: 2267 End: 2491 poly A
10	poly A
	Start: 4343 End: 4473 SV40 poly A signal
	Promoter Eukaryotic (1 total)
	promoter
	Start: 232 End: 819 eukaryotic promoter
15	Promoter Prokaryotic (1 total)
	promoter
	Start 6566 End: 6572 (Complementary) promoter
	Replication Origin (3 total)
	SV40 promoter and origin
20	Start 1 End: 1 origin of replication
	F1 origin
	Start: 2537 End: 2965 origin of replication
	pUC origin
	Start 4856 End: 5526 (Complementary) origin
25	

**Table 2: pB11sfv-βhCG Feature Map**

	CDS (4 total)
	Light Chain
	Start 735 End: 1433 B11 Light Chain
30	C kappa
	Start: 1113 End: 1433 AMP
	Start: 7810 End: 8670 (Complementary) amp
	Original Location Description: complemented 1 ..6871)
	DHFR
35	Start: 8921 End: 9484 dhfr
	Original Location Description: 7122-7685
	Misc. Feature (9 total)
	B11 VL
	Start: 792 End: 1112 SV40 Promoter/Ori
40	Start 2298 End: 2622
	SV40 promoter and origin of replication

- Neo  
 Start: 2658 End: 3452 Neomycin Resistance Gene  
 beta HCG  
 Start: 4015 End: 4467 (Complementary) bHCG
- 5 CHS  
 Start: 4470 End: 4790 (Complementary) Heavy chain constant region 3  
 CH2  
 Start: 4791 End: 5120 (Complementary) Heavy chain constant region 2  
 CH1
- 10 Start 5166 End: 5459 (Complementary) heavy chain constant region 1  
 B11 VH  
 Start: 5460 End: 5807 (Complementary) Promoter  
 Start: 5905 End: 6559 (Complementary)
- PolyA Signal (3 total)
- 15 Poly A  
 Start: 1526 End: 1757 PolyA  
 Start: 3744 End: 3975 (Complementary) PolyA\_Signal\_2  
 Start 10282 End: 10411 SV40 poly A  
 Original Location Description: 8483..8612
- 20 Promoter Eukaryotic (1 total)  
 Promoter  
 Start 9 End: 655

Chemically constructed antibody-antigen conjugates can be made using a

25 variety of well known and readily available cross-linking reagents. These cross-linking reagents can be homofunctional or heterofunctional compounds, such as N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl-S-acetyl-thioacetate (SATA), sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), that form covalent linkages with

30 different reactive amino acid or carbohydrate side chains on the anti-dendritic antibody and selected antigen. Other coupling and cross-linking agents also can be used to generate covalent linkages, such as protein A, carbodiimide, and o-phenylenedimaleimide (oPDM); (see *e.g.*, Karpovsky *et al.* (1984) J. Exp. Med. 160:1686; Liu, MA *et al.* (1985) Proc. Natl. Acad. Sci. USA 82:8648). Other methods

35 include those described by Paulus (Behring Ins. Mitt. (1985) No. 78, 118-132); Brennan *et al.* (Science (1985) 229:81-83), and Glennie *et al.* (J. Immunol. (1987) 139: 2367-2375). Preferred conjugating agents are SATA and sulfo-SMCC, both available from Pierce Chemical Co. (Rockford, IL). Immunostimulatory agents can also be chemically linked to the molecular conjugates of the present invention using the same linking

40 methods described above.

Immunostimulatory agents and molecular conjugates can also be linked via non-covalent methods, for example, using binding molecules such as streptavidin and biotin. Other suitable binding molecules for use in the present invention are well-known in the art. In one aspect of the invention, the nucleotide sequence encoding a streptavidin molecule is incorporated into the sequence encoding the molecular conjugate which, in turn, is linked to a biotinylated immunostimulatory agent.

Any antigen that can be cloned and expressed or purified can be selected for use in the present invention. Techniques for obtaining such antigens are well-known in the art. For example, tumor-associated antigens can be directly purified from cancer cells and identified by physiochemical techniques such as tandem mass spectrometry. Alternatively, tumor-specific T-cell clones can be tested against antigen-negative cells that have acquired antigen by being transfected with plasmid DNA clones to isolate the clone expressing the antigen. Synthetic peptides can then be constructed to precisely identify the antigenic site or epitope.

As discussed above, the molecular vaccine conjugates of the invention can be administered together with, or include, one or more immunostimulatory agents. The immunostimulatory agent can be administered separately or can be linked to the conjugate, either covalently, non-covalently, genetically, or a combination thereof, according to the linking techniques discussed above. Alternatively, the immunostimulatory agent can be co-administered separately, for example, the agent can be administered simultaneously with the molecular conjugate, or prior to administration of the molecular conjugate, or subsequent to administration of the molecular conjugate. A variety of suitable immunostimulatory agents are well known in the art and include, for example, CD40 ligand; cytokines, such as IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$  and IL-2; colony-stimulating factors, such as G-CSF (granulocyte colony-stimulating factor) and GM-CSF (granulocyte-macrophage colony-stimulating factor); an anti-CTLA-4 antibody; toll receptor agonists (*e.g.*, flagellin and MALP-2 (macrophage activating lipopeptide-2); LPS (endotoxin); R837 (3M Pharmaceuticals, St. Paul, MN); R848 (3M Pharmaceuticals, St. Paul, MN); polyI:C (inosine:cytosine polynucleotide); ssRNA; dsRNA; Bacille Calmette-Guerin (BCG); Levamisole hydrochloride; and intravenous immune globulins.

In another aspect of the invention, partial antibody sequences from the vaccine construct can be used to express intact antibodies. Antibodies, such as the anti-APC antibodies (*e.g.*, B11) encompassed by the vaccine conjugates of the present invention, interact with target antigens (*e.g.*, C-type lectin receptors, such as the MR) predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences



outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, *e.g.*, Riechmann, L. *et al.* (1998) *Nature* 332:323-327; Jones, P. *et al.* (1986) *Nature* 321:522-525; and Queen, C. *et al.* (1989) *Proc. Natl. Acad. Sci. U.S.A.* 86:10029-10033). Such framework sequences can be obtained from public DNA databases that include germline antibody gene sequences. These germline sequences will differ from mature antibody gene sequences because they will not include completely assembled variable genes, which are formed by V(D)J joining during B cell maturation. Germline gene sequences will also differ from the sequences of a high affinity secondary repertoire antibody at individual evenly across the variable region. For example, somatic mutations are relatively infrequent in the amino-terminal portion of framework region. For example, somatic mutations are relatively infrequent in the amino terminal portion of framework region 1 and in the carboxy-terminal portion of framework region 4. Furthermore, many somatic mutations do not significantly alter the binding properties of the antibody. For this reason, it is not necessary to obtain the entire DNA sequence of a particular antibody in order to recreate an intact recombinant antibody having binding properties similar to those of the original antibody (see WO 99/45962, which is herein incorporated by referenced for all purposes). Partial heavy and light chain sequence spanning the CDR regions is typically sufficient for this purpose. The partial sequence is used to determine which germline variable and joining gene segments contributed to the recombined antibody variable genes. The germline sequence is then used to fill in missing portions of the variable regions. Heavy and light chain leader sequences are cleaved during protein maturation and do not contribute to the properties of the final antibody. For this reason, it is necessary to use the corresponding germline leader sequence for expression constructs. To add missing sequences, cloned cDNA sequences can be combined with synthetic oligonucleotides by ligation or PCR amplification. Alternatively, the entire variable region can be synthesized as a set of short, overlapping, oligonucleotides and combined by PCR amplification to create an entirely synthetic variable region clone. This process has certain advantages such as elimination or inclusion of particular restriction sites, or optimization of particular codons.

The nucleotide sequences of heavy and light chain transcripts from hybridomas are used to design an overlapping set of synthetic oligonucleotides to create synthetic V sequences with identical amino acid coding capacities as the natural sequences. The synthetic heavy and kappa chain sequences can differ from the natural sequences in three ways: strings of repeated nucleotide bases are interrupted to facilitate

oligonucleotide synthesis and PCR amplification; optimal translation initiation sites are incorporated according to Kozak's rules (Kozak (1991) J. Biol. Chem. 266:19867-19870); and HindIII sites are engineered upstream of the translation initiation sites.

For both the heavy and light chain variable regions, the optimized coding, and corresponding non-coding, strand sequences are broken down into 30-50 nucleotide approximately the midpoint of the corresponding non-coding oligonucleotide. Thus, for each chain, the oligonucleotides can be assembled into overlapping double stranded sets that span segments of 150-400 nucleotides. The pools are then used as templates to produce PCR amplification products of 150-400 nucleotides. Typically, a single variable region oligonucleotide set will be broken down into two pools which are separately amplified to generate two overlapping PCR products. These overlapping products are then combined by PCR amplification to form the complete variable region. It may also be desirable to include an overlapping fragment of the heavy or light chain constant region (including the BbsI site of the kappa light chain, or the AgeI site of the gamma heavy chain) in the PCR amplification to generate fragments that can easily be cloned into the expression vector constructs.

The reconstructed heavy and light chain variable regions are then combined with cloned promoter, translation initiation, constant region, 3' untranslated, polyadenylation, and transcription termination, sequences to form expression vector constructs. The heavy and light chain expression constructs can be combined into a single vector, co-transfected, serially transfected, or separately transfected into host cells which are then fused to form a host cell expressing both chains.

Plasmids for use in construction of expression vectors for human IgG<sub>k</sub> are described below. The plasmids were constructed so that PCR amplified V heavy and V kappa light chain cDNA sequences could be used to reconstruct complete heavy and light chain minigenes. These plasmids can be used to express completely human, or chimeric IgG<sub>1k</sub> or IgG<sub>4k</sub> antibodies. Similar plasmids can be constructed for expression of other heavy chain isotypes, or for expression of antibodies comprising lambda light chains.

Thus, in another aspect of the invention, the structural features of the antibody portion of the vaccine conjugates described herein, *e.g.*, B11, are used to create structurally related antibodies that retain at least one functional property of the B11 antibody of the invention, such as binding to APCs. More specifically, one or more CDR regions of B11 can be combined recombinantly with known human framework regions and CDRs to create additional, recombinantly-engineered, anti-APC antibodies for use in the vaccine conjugates of the invention.

Accordingly, in another embodiment, the invention provides a method for preparing a vaccine conjugate comprising an anti-DC antibody comprising: preparing an antibody comprising (1) human heavy chain framework regions and human heavy chain CDRs, wherein at least one of the human heavy chain CDRs comprises an amino acid  
5 sequence selected from the amino acid sequences of CDRs shown in Figure 8 (SEQ ID NOs:13, 14, or 15); and (2) human light chain framework regions and human light chain CDRs, wherein at least one of the human light chain CDRs comprises an amino acid sequence selected from the amino acid sequences of CDRs shown in Figure 9 (SEQ ID NO:16, 17, or 18); wherein the antibody retains the ability to bind to APCs.

10 The ability of the antibody to bind APCs can be determined using standard binding assays, such as those set forth in the Examples (*e.g.*, an ELISA). Since it is well known in the art that antibody heavy and light chain CDR3 domains play a particularly important role in the binding specificity/affinity of an antibody for an antigen, the recombinant antibodies of the invention prepared as set forth above  
15 preferably comprise the heavy and light chain CDR3s of B11. The antibodies further can comprise the CDR2s of B11. The antibodies further can comprise the CDR1s of B11. Accordingly, the invention further provides anti-APC antibodies comprising: (1) human heavy chain framework regions, a human heavy chain CDR1 region, a human heavy chain CDR2 region, and a human heavy chain CDR3 region, wherein the human  
20 heavy chain CDR3 region is the CDR3 of B11 as shown in Figure 8 (SEQ ID NO:15); and (2) human light chain framework regions, a human light chain CDR1 region, a human light chain CDR2 region, and a human light chain CDR3 region, wherein the human light chain CDR3 region is the CDR3 of B11 as shown in Figure 9 (SEQ ID NO: 18), wherein the antibody binds DC. The antibody may further comprise the heavy  
25 chain CDR2 and/or the light chain CDR2 of B11. The antibody may further comprise the heavy chain CDR1 and/or the light chain CDR1 of B11.

Preferably, the CDR1, 2, and/or 3 of the engineered antibodies described above comprise the exact amino acid sequence(s) as those of B11 disclosed herein. However, the ordinarily skilled artisan will appreciate that some deviation from the  
30 exact CDR sequences of B11 may be possible while still retaining the ability of the antibody to bind DC effectively (*e.g.*, conservative substitutions). Accordingly, in another embodiment, the engineered antibody may be composed of one or more CDRs that are, for example, at least 90%, 95%, 98% or 99.5% identical to one or more CDRs of B11.

35 In addition or alternatively to simply binding APCs, engineered antibodies such as those described above may be selected for their retention of other functional properties of antibodies of the invention, such as:

- (1) high affinity binding to APCs;
- (2) binding to a unique epitope on an APC (to eliminate the possibility that monoclonal antibodies with complimentary activities when used in combination would compete for binding to the same epitope);
- 5 (3) induces a T cell-mediated immune response which is generated against the antigen; and/or
- (4) induces a T cell response which comprises both CD4<sup>+</sup> and CD8<sup>+</sup> T cell-mediated responses.

10 In another embodiment, a whole cell expressing the antigen of interest, *e.g.*,  $\beta$ hCG, is transformed to express an anti-APC antibody, *e.g.*, an anti-MR antibody, so that the antigen and the antibody are co-expressed by the cell. This can be done, for example, by transfecting the target cell with a nucleic acid encoding a fusion protein containing a transmembrane domain and an anti-APC antibody. The cell expressing the vaccine conjugate can then be used to target APCs, *e.g.*, DCs, to induce a CTL response.

15 Methods for generating such nucleic acids, fusion proteins, and cells expressing such fusion proteins are described, for example, in U.S. Patent Application Serial No. 09/203,958, incorporated herein in its entirety by this reference.

20 Alternatively, the antibody can be bound to a cell or a pathogen by the use of chemical linkers, lipid tags, or other related methods (deKruif, J. *et al.* (2000) *Nat. Med.* 6:223-227; Nizard, P. *et al.* (1998) *FEBS Lett.* 433:83-88). Cells which express the antigen of interest and with surface-anchored antibodies may be used to induce specific immune responses, *e.g.*, a CTL response, against the cell, *e.g.*, a tumor cell or microbial pathogen.

### 25 III. Pharmaceutical Compositions

In another aspect, the present invention provides therapeutic compositions, *e.g.*, pharmaceutical compositions, containing one or a combination of vaccine conjugates of the present invention formulated together with an immunostimulatory agent. In one embodiment, the immunostimulatory agent is linked  
30 to the vaccine conjugate. The compositions of the present invention may further include one or more adjuvants and/or pharmaceutically acceptable carrier. The vaccine conjugate of the present invention is administered for delivery into the subject's bloodstream for interaction with the subject's T cells. Such targeting of T cells can be accomplished either *in vivo* or *ex vivo* by directly using the conjugate or by using cells  
35 which have been previously been targeted with vaccine conjugates.

The compositions of the present invention can additionally include other therapeutic reagents, such as other antibodies, cytotoxins or drugs (*e.g.*, immunosuppressants), and can be administered alone or in combination with other

therapies, such as radiation. For example, a vaccine conjugate that is rapidly internalized by APCs can be combined with a monoclonal antibody that enhances antigen presenting cell activities of dendritic cells, *e.g.*, release of immunostimulatory cytokines.

5                   As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of  
10 administration, the vaccine conjugate may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired  
15 toxicological effects (see *e.g.*, Berge, S.M., et al. (1977) J. Pharm. Sci. 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-  
20 substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chlorprocaine, choline, diethanolamine, ethylenediamine, procaine  
25 and the like.

Compositions of the present invention can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. The active compounds can be prepared with carriers that will protect the compound against rapid  
30 release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g.*,  
35 *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

To administer a vaccine conjugate of the invention by certain routes of administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a  
5 diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan *et al.* (1984) *J. Neuroimmunol.* 7:27).

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable  
10 solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

15 Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid  
20 polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition.  
25 Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of  
30 ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-  
35 drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

For the therapeutic compositions, formulations of the present invention include those suitable for oral and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.01 per cent to about ninety-nine percent of active ingredient, preferably from about 0.1 per cent to about 70 per cent, most preferably from about 1 per cent to about 30 per cent.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given alone or as a pharmaceutical composition containing, for example, 0.01 to 99.5% (more preferably, 0.1 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.



A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that  
5 required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a compositions of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. It is preferred that administration be  
10 intravenous, intramuscular, intraperitoneal, or subcutaneous, preferably administered proximal to the site of the target. If desired, the effective daily dose of a therapeutic compositions may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. While it is possible for a compound of the present invention to be  
15 administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition).

Therapeutic compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of the invention can be administered with a needleless hypodermic injection device, such as  
20 the devices disclosed in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which discloses a therapeutic device for administering  
25 medicants through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196,  
30 which discloses an osmotic drug delivery system. These patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

The composition must be sterile and fluid to the extent that the composition is deliverable by syringe. In addition to water, the carrier can be an isotonic  
35 buffered saline solution, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by use of coating such as lecithin, by maintenance of required particle size in the case of dispersion and by use of surfactants. In many cases,

it is preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol or sorbitol, and sodium chloride in the composition. Long-term absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

- 5                   When the active compound is suitably protected, as described above, the compound may be orally administered, for example, with an inert diluent or an assimilable edible carrier.

#### IV. Uses and Methods of the Invention

- 10                   Vaccine conjugates of the present invention can be used to treat and/or prevent (*e.g.*, immunize against) a variety of diseases and conditions.

- One of the primary disease indications is cancer. This includes, but is not limited to, colon cancer, melanoma, lymphoma, prostate carcinoma, pancreatic carcinoma, bladder carcinoma, fibrosarcoma, rhabdomyosarcoma, mastocytoma, 15 mammary adenocarcinoma, leukemia, or rheumatoid fibroblastsoma. Another primary disease indication is infectious diseases including, but not limited to, HIV, Hepatitis (*e.g.*, A, B, & C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus Aureus, Pseudomonas aeruginosa. Another primary disease indication is autoimmune diseases.

- 20                   In a particular embodiment, the vaccine conjugates are used to treat or prevent diseases and conditions mediated by  $\beta$ hCG or cells expressing  $\beta$ hCG, which is a member of the cysteine-loop growth factor superfamily. Evidence suggests that  $\beta$ hCG plays a role in the establishment or progression of cancers either as a growth factor, as an angiogenesis and/or metastasis-promoting agent, or as a suppressor of immune 25 function (73). Accordingly, the present invention can be used to treat the progression of cancers and other diseases involving angiogenesis. The invention also can be used to prevent or terminate unwanted pregnancy by inhibiting the role of  $\beta$ hCG and/or cells expressing  $\beta$ hCG in pregnancy.

- For use in therapy, vaccine conjugates of the invention can be 30 administered to a subject directly (*i.e.*, *in vivo*), either alone or with an immunostimulatory agent. In one aspect, the immunostimulatory agent is linked to the conjugate. Alternatively, the conjugates can be administered to a subject indirectly by first contacting the conjugates (*e.g.*, by culturing or incubating) with APCs, such as dendritic cells, and then administering the cells to the subject (*i.e.*, *ex vivo*). The 35 contacting and delivering of the conjugates to APCs, such that they are processed and presented by the APCs prior to administration, is also referred to as antigen or cell "loading." Techniques for loading antigens to APCs are well known in the art and

include, for example, Gunzer and Grabbe, Crit Rev Immunol 21 (1-3):133-45 (2001) and Steinman, Exp Hematol 24(8): 859-62 (1996).

In all cases, the vaccine conjugates and the immunostimulatory agents are administered in an effective amount to exert their desired therapeutic effect. The term  
5 "effective amount" refers to that amount necessary or sufficient to realize a desired biologic effect. For example, an effective amount could be that amount necessary to eliminate a tumor, cancer, or bacterial, viral or fungal infection. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular conjugate being administered, the size of the  
10 subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular multispecific molecule without necessitating undue experimentation.

Preferred routes of administration for the vaccine conjugates include, for example, injection (*e.g.*, subcutaneous, intravenous, parenteral, intraperitoneal,  
15 intrathecal). The injection can be in a bolus or a continuous infusion. Other routes of administration include oral administration.

Vaccine conjugates of the invention also can be coadministered with adjuvants and other therapeutic agents, such as immunostimulatory agents. The conjugates are typically formulated in a pharmaceutically acceptable carrier alone or in  
20 combination with such agents. Examples of such carriers include solutions, solvents, dispersion media, delay agents, emulsions and the like. The use of such media for pharmaceutically active substances are well known in the art. Any other conventional carrier suitable for use with the molecules falls within the scope of the instant invention.

Suitable agents for coadministration with the vaccine conjugates include  
25 other antibodies, cytotoxins and/or drugs. In one embodiment, the agent is a anti-CTLA-4 antibody which are known to aid or induce immune responses. In another embodiment, the agent is a chemotherapeutic agent. The vaccine conjugates also can be administered in combination with radiation.

30 The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

## EXAMPLES

## Methods and Materials

5                    Generation of DCs from whole blood or leukopak: Human peripheral  
blood mononuclear cells (PBMC) were obtained by density gradient centrifugation of  
heparinized whole blood or apheresis preparations with Ficoll-Paque. Monocytes were  
then isolated by adherence to plastic culture dishes or elutriation and differentiated into  
immature DCs by addition of cytokines (10 ng/ml GM-CSF and 2 ng/ml IL-4) to the  
10 culture medium. DCs were harvested between day 5 and 7 and analyzed by flow  
cytometry. The DCs prepared in this fashion were CD14<sup>-</sup>, HLA-DR<sup>+</sup>, CD11c<sup>+</sup> mannose  
receptor<sup>+</sup> and expressed high levels of MHC Class I and II, CD80 and CD86.

**Selection of tumor antigen  $\beta$ hCG:**  $\beta$ hCG is a subunit of human chorionic gonadotropin, a hormone necessary for the establishment of a successful pregnancy. This glycoprotein subunit has a number of features that make it an attractive antigen for cancer immunotherapy (reviewed in Triozzi P.L. and Stevens V. (1999) *Oncology Reports* 6:7-17). First, aside from pregnancy, the expression of this antigen is primarily restricted to germ cell tumors, as well as a significant number of adenocarcinomas (Table 3). Also, hCG is a member of the cysteine-loop growth factor superfamily and may play a role in the establishment or progression of cancers either as a growth factor, an angiogenesis and/or metastasis-promoting agent, or as a suppressor of immune function. Immunotherapy that limits the expression of functional hCG may therefore offer added therapeutic benefit.

Table 3

Percent of tumors positive for  $\beta$ hCG by immunohistochemistry (Triozi P.L. and Stevens V. (1999)).

Colon (52%)	Bladder (21%)
Lung (34%)	Ovary (19%)
Pancreas (31%)	Cervix (18%)
Esophagus (28%)	Gastric (18%)
Breast (24%)	

30 Proliferation Assay: Effector T cells ( $5 \times 10^4$ ) were co-cultured with autologous DCs ( $5 \times 10^3$ ) loaded with or without antigen (MDX-1307 or other) in 96 well flat bottomed microplates in 0.2 ml final volume. The mixture was cocultured at 37°C. On day 4, cultures were pulsed with  $^3\text{H}$ -thymidine (1  $\mu\text{Ci}/\text{well}$ ) and 18 hours later, cells

were harvested directly on filters (Millipore). Filters were washed three times with water followed by one wash in ethanol and allowed to dry under the hood for 5-10 min. Scintillation fluid (Packard, 20  $\mu$ l/well) was then added to the filters. Filter-bound radioactivity was determined by counting on the Wallac beta counter. The results are expressed as stimulation index (S.I.) values in cpm of CTL stimulated with antigen versus stimulation with no antigen or control antigen. For MHC blocking analysis, labeled targets were preincubated with HLA-specific mAbs, W6/32 for blocking all class I and L243 for blocking all class II HLA molecules (20  $\mu$ g/ml), for 30 min. at RT. Unbound mAb was removed by centrifugation.

10

Flow cytometry: Human DCs were prepared from monocytes by culture in GM-CSF and IL-4 for 5 days. DCs were incubated on ice with 10  $\mu$ g/ml of the  $\beta$ hCG antigen/anti-MR antibody vaccine conjugate or an isotype control. Vaccine conjugates were either directly FITC-labeled or detected with an FITC-labeled anti- $\beta$ hCG secondary monoclonal antibody. The cell associated fluorescence was determined using an LSR flow cytometer.

Cytotoxicity Assay: Target cells ( $3 \times 10^6$ ), control and antigen loaded ( $\beta$ hCG-B11), were washed twice in RPMI medium and the pellet was resuspended in 200  $\mu$ l medium and labeled with 100  $\mu$ Ci  $^{51}\text{Na}_2\text{CrO}_4$  for 60 min at 37°C. Labeled targets were washed 3 times in RPMI medium and the pellet resuspended to yield a cell concentration of  $3 \times 10^4$  cells/ml. Antigen-specific CTL were titrated in a 96 well V-bottomed plate to give ratios of 100:1 (effector T cell, E: target, T) through to 12.5: 1 or lower. A constant number of labeled targets were added (100  $\mu$ l/well or 3,000 target cells/well) and the plates were spun down at low speed (180 x g) and incubated at 37°C. After 4 hours, 100-120  $\mu$ l supernatant was harvested and the radioactivity released was determined in a  $\gamma$ -counter counting (Wallac Instruments, Perkin-Elmer). CTL activity was calculated and expressed as % Specific Lysis (killing) using the following equation:

$$\text{Specific Lysis (\%)} = \frac{\text{Experimental Release (cpm)} - \text{Spontaneous Release (cpm)}}{\text{Maximal Release (cpm)} - \text{Spontaneous Release (cpm)}} \times 100 ;$$

where Experimental (cpm) refers to radioactivity (chromium released) from wells containing CTL (E) and target (T); Spontaneous (cpm) refers to the radioactivity from wells with targets in 0.1 ml medium alone (*i.e.* no CTL added) while Maximal release refers to radioactivity from wells with targets in the presence of 0.1 ml detergent solution (Igepal CA 630; syn. NP-40; 5% solution in RPMI medium). Under well-controlled experimental conditions, Spontaneous release values should be 10% of Maximal release or less. For MHC blocking analysis, labeled targets were preincubated

with HLA-specific mAbs, W6/32 for blocking all class I and L243 for blocking all class II HLA molecules (20 µg/ml) for 30 min. at RT. Unbound mAb was removed by centrifugation and mAb-coated targets were added to CTL. An isotype-matched mAb was used as a control.

- 5 Yet another way to look at cell-mediated immune responses is to investigate the proliferative capacity of antigen-driven T cells. Antigen-sensitized T cells tend to proliferate preferentially when previously exposed antigens are presented in the context of MHC class II and to a lesser extent, class I molecules. Thus, the enumeration of dividing cells by uptake of a radioactive tracer provides a measure of stimulation.
- 10

- In vivo antigen distribution: Mice were injected subcutaneously (s.c.) in the two front paws with 5 µg of B11-βhCG in 5 µl PBS to each side. The same amount of irrelevant human IgG1 (Sigma-Aldrich) was administered via the same route as a non-targeting control. Skin around and remote from the injection site, proximal (axillary) and distal (inguinal) lymph nodes, spleen, liver and lung were harvested 24 hours later. These organs were snap frozen and sectioned for immunohistochemical staining with antibodies against hCG (antigen) or human IgG (to detect B11).
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- Immunization: For humoral responses, mice of 8-12 weeks of age were immunized i.p. with B11-βhCG in 100 µl PBS per mouse at 2-week interval, 3 doses in total. Equivalent amount of free βhCG (United States Biological, Swampscott, MA), were injected with same formulation and time course as another arm of comparison. Each study comprised 5-6 transgenic (tg) mice and same number of age-, gender-
- 20 matched wildtype (Wt) mice. Blood specimens were collected from the orbital sinus under anesthetics on day 0 for baseline and 1 week after each injection and plasma were isolated and stored at -20°C for ELISA.
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- For T cell responses, mice 8-12-week-old were administered once via i.p. with 1 or 2 µg of B11-βhCG in 100 µl of adjuvants (CPG and Poly:IC). Each study comprised 2-3 tg mice and Wt mice, respectively. Some of these studies were repeated to confirm the results. Blood and spleen were harvested from these mice 14 days after injection. Plasmas isolated from these bloods were stored at -20° C for antibody subclass analysis.
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- ELISA: Anti-βhCG and anti-B11 titers were measured by standard ELISA with immobilized βhCG or B11 for capture and alkaline phosphatase (AP)-conjugated goat anti-mouse IgG Fcy antibody (Jackson ImmunoResearch Lab, West Grove, PA) for detection. Plasma specimen was diluted serially starting from 1:100, and
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titers were determined by calculating the dilution of each specimen giving an absorbance at OD<sub>405</sub> that was above twice that of un-immunized mouse serum control. Antibody subclass determination was done by means of ELISA with SBA Clonotyping System/AP (SouthernBiotechnology Associates, Birmingham, AL).

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ELISOPT: Assay was performed with ELISPOT sets (BD Biosciences) following the manufacturer's protocol. Single splenocyte suspension was prepared by grinding spleen and lysing red blood cells.  $0.5 \times 10^6$  or  $1 \times 10^6$  cells per well in 96-well filtration plates coated with anti-IFN $\gamma$  or IL-4 antibodies were cultured in RPMI 1640 complete medium. 15-mer  $\beta$ hCG overlapping peptide pool covering the entire  $\beta$ hCG sequence, synthesized by Synpep Corporation (Dublin, CA), was added to the culture to a final concentration of 1.7  $\mu$ g/ml and incubated at 37° C for 40 hr. Wells at absence of the peptide pool (blank) served as non-specific background control. Wells containing 10 ng/ml of PMA plus 1  $\mu$ g/ml of Ionomycin (Sigma-Aldrich) served as positive control for spots development assay and immunocompetence of the mice. Each condition of treatment was set up in triplicate for each sample. Plates were analyzed by ZellNet Consulting, Inc. (Fort Lee, NJ). Antigen  $\beta$ hCG-specific spot number of each sample was mean $\pm$ SD of the triplicate of peptides-treated wells after subtracting numbers in blank controls.

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### **Example 1    Production of $\beta$ hCG-B11**

Design of vaccine conjugate: This construct was generated by linking the  $\beta$ hCG antigen to B11, a fully human antibody which binds to the human macrophage mannose receptor on dendritic cells. Linkage was accomplished by covalently attaching the antigen to the heavy chain of the antibody by way of a genetic fusion, as shown in Figure 3.

Recombinant Expression of  $\beta$ hCG-B11 Vaccine Conjugate: As shown in Figure 2, a plasmid containing neomycin and dihydrofolate reductase genes was generated containing the  $\beta$ hCG coding sequence fused to antibody B11 at the CH<sub>3</sub> domain of the heavy chain (SEQ ID NOs:9 and 10). The resulting plasmid construct was transfected into CHO cells using a standardized protocol (Qiagen Inc, Valencia, CA). Transfected cells were selected in media containing the antibiotic G418. Expression was further amplified by growing cells in increasingly higher concentrations of methatrexate. After amplification, the cells were cloned by limiting dilution, and stable clonal lines were used to generate cell banks for further studies. To confirm expression of the  $\beta$ hCG-B11 constructs, Western Blot analysis of proteins run on SDS-PAGE under

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reducing conditions was performed. This fusion protein was observed to be of the expected molecular weight and to be properly assembled (*i.e.*, to contain both the heavy chain fusion and the light chain). Specifically, the vaccine conjugate and the antibody alone were analyzed by SDS- PAGE using denaturing conditions and detected by Western blot analysis. The blot was then probed separately using goat anti-human IgG heavy and light, and with a mAb (Sigma) specific to the  $\beta$ hCG C-terminal peptide. The results confirmed that the transformed CHO cells specifically expressed the B11- $\beta$ hCG vaccine conjugate as evidenced by the appropriate size and composition of the fusion product.

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## **Example 2    Production of B11 scfv - $\beta$ hCG**

Design of vaccine conjugate: A second construct was generated by linking the  $\beta$ hCG antigen to a B11 single chain fusion (ScFv), which is a single chain antibody that binds to the human macrophage mannose receptor on dendritic cells and contains the  $V_L$  and  $V_H$  fragments of the fully human B11 antibody. Linkage was accomplished by covalently attaching the antigen to the carboxy terminus of the B11 ScFv by way of a genetic fusion, as shown in Figure 1 (referred to as the B11sfv- $\beta$ hCG construct).

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Recombinant Expression of B11sfv- $\beta$ hCG Vaccine Conjugate: As shown in Figure 1, a plasmid was generated containing the B11sfv- $\beta$ hCG construct (SEQ ID NOs: 11 and 12). The resulting plasmid construct was transfected into mammalian cells using a standardized protocol (Qiagen Inc, Valencia, CA). Transfected cells were selected in media containing the antibiotic G418. An ELISA was performed to confirm expression of the B11sfv- $\beta$ hCG construct.

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## **Example 3    Functional characterization of vaccine conjugates**

Antibody-targeted vaccine recognition of its cognate receptor on the APC surface is the first step in this delivery platform. Flow cytometry studies have been used to demonstrate that the  $\beta$ hCG-B11 and B11sfv- $\beta$ hCG constructs bind specifically to cultured human DC expressing MR (Figure 4).

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Using the anti-MR antibody as a probe, *in situ* staining of MR on human dermal DCs and macrophages in section of various human tissues was examined. Human tissue cryosections were stained with anti-MR human antibody B11. DCs present in the dermal layer of the skin were clearly labeled (data not shown) with the B11 antibody. It is noted that there was binding to DCs in the dermal layer of skin.

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Furthermore, immunohistochemistry performed with the anti-MR B11 HuMAb stained dendritic cells in all tissues tested and showed no unexpected cross-reactivity (results not shown). These studies have been repeated with the  $\beta$ hCG-B11 with identical results.

**5 Example 4 Cross-presentation of the  $\beta$ hCG antigen/anti-MR antibody vaccine conjugate to T cells**

The capacity of the  $\beta$ hCG-B11 construct to be processed by DCs for presentation of  $\beta$ hCG antigen to T cells *via* MHC class I and class II molecules on DCs  
 10 (cross-presentation) was evaluated. In particular, the  $\beta$ hCG-B11 construct was used to elicit antigen-specific T cells by culturing a pool of normal T cells with DCs that were exposed to the vaccine. The resulting "sensitized" T cells were then analyzed for their activity (proliferation and killing) and specificity. Specificity of the T cells can be demonstrated by comparing the T cell activity in response to target cells that have the  
 15  $\beta$ hCG antigen to antigen-negative controls. Cytotoxic T cells (CTL), if present, should kill only those targets that present  $\beta$ hCG related antigen but spare control targets that are either lacking the antigen or presenting an unrelated antigen. Since CTL-mediated antigen recognition always occurs in the context of a given MHC molecule bearing the peptide, blocking the MHC:peptide-CTL interaction with an MHC-specific mAb  
 20 confirms the class I or class II presentation.

Induction of antigen-specific effector T cells: Dendritic cells were generated from normal donor peripheral blood mononuclear cells (PBMC) by culturing adherent monocytes with 25 ng/ml recombinant human GM-CSF (R&D systems, MN)  
 25 and 100 ng/ml of recombinant human IL-4 for 5 days. On day 5, DCs were harvested (immature) and resuspended in AIM-V (serum-free) medium. The  $\beta$ hCG-B11 immunoconjugate (20  $\mu$ g/ml) was added to  $1.2 \times 10^6$  DC and incubated for 45 min at 37°C. Antigen-loaded DCs was allowed to mature in the presence of CD40L (Peprotech, NJ; 20 ng/ml) for at least 24 hours. Mature DC ( $1 \times 10^6$ ) were washed once  
 30 and added to T cells ( $2 \times 10^7$ ; bulk) previously seeded in 24 well plates at  $1 \times 10^6$  cells/ml (ratio of DC: T cells, 20). The following culture conditions were employed: addition of 10 ng/ml IL-7 on day 0, followed by 10 ng/ml of IL-10 on day 1 (at 24 hours), and 20 U/ml IL-2 on day 2 (at 48 hours). Restimulation was carried out on days 7, 14 and 21 as before, except that  $\beta$ hCG-B11 concentration was cut by half (10, 5 and  
 35 2.5  $\mu$ g/ml, respectively). T cells were tested for reactivity (either in bulk or with purified T cell sub populations) against  $^{51}$ Cr-labeled DC loaded with nothing,  $\beta$ hCG-B11, B11sfv- $\beta$ hCG, or B11. MHC-specificity was ascertained in the presence of HLA-specific mAbs.

As illustrated in Figure 5, the  $\beta$ hCG-B11 construct induced  $\beta$ hCG-specific cytotoxic T cells. No killing ensued if the T cells were cultured with targets that do not present  $\beta$ hCG. The target cells used in these experiments were HLA-matched DC treated with the  $\beta$ hCG-B11 construct or control antigens. Target cells treated only with the anti-MR antibody (B11) were not susceptible to the cytotoxic activity, demonstrating that only the antigen portion of the vaccine was able to elicit CTL activity. These results show that the  $\beta$ hCG-B11 construct induces efficient CTL activity and, specifically, the CTL activity is directed towards the  $\beta$ hCG antigen but not the targeting antibody (B11).

Furthermore, the potent killing of targets presenting  $\beta$ hCG antigen was reproduced with purified  $CD8^+$  T cells, which killing was blocked in the presence of anti-MHC class I antibodies (Figure 6). In particular, the  $\beta$ hCG-B11 construct was used to generate  $\beta$ hCG-specific T cells from peripheral blood mononuclear cells of two donors.  $CD8^+$  and  $CD4^+$  T cells were purified from bulk cultures using immunomagnetic beads. Cytotoxicity assays were carried out as described above with the effector:target ratio set at 40:1. The target cells (immature DC) were untreated (control) or loaded with the  $\beta$ hCG-B11 construct. To demonstrate MHC Class I specificity, target cell killing was blocked by preincubation with an HLA-specific antibody (W6/32).

Collectively, these data (Figures 6 and 7) confirm the ability of the  $\beta$ hCG-B11 construct to induce potent  $\beta$ hCG-specific CTL, and additionally demonstrate that the CTL activity is mediated by  $CD8^+$  T cells in an HLA-dependent manner. No killing activity was observed with the purified  $CD4^+$  T cells.

As shown in Figure 7, the  $\beta$ hCG-B11 construct -elicited T cells proliferate in response to the  $\beta$ hCG-B11 construct targeted DC. In particular, DC were treated with the  $\beta$ hCG-B11 construct to generate  $\beta$ hCG-specific T cells from peripheral blood mononuclear cells. T cells from bulk cultures ( $CD4^+$  and  $CD8^+$  T cells) were tested for proliferation in response to antigen stimulation. T cells were co-cultured with untreated DC (control) or DC loaded with the  $\beta$ hCG-B11 construct with or without HLA blocking antibodies. To measure proliferation, DNA synthesis was analyzed after 5 days of culture using  $^3H$ -thymidine. The data were expressed as the fold-increase in proliferation (stimulation index) over control. As seen with the CTL activity, no appreciable response was found when the T cells were stimulated by DC alone (*i.e.*, no antigen). DC targeted with only the unconjugated antibody (anti-MR B11 mAb) did not induce proliferation of T cells elicited by the  $\beta$ hCG - B11 construct. The proliferative capacity of the T cells was significantly blocked in the presence of both anti-MHC class I as well as class II-specific mAbs, demonstrating that both  $CD4^+$  and  $CD8^+$  T cells were responding. These data show that the uptake of the  $\beta$ hCG-B11 construct by DC enables

the vaccine to gain access to MHC class I and class II processing pathways, which is consistent with co-localization of MR with MHC compartments.

**Example 5     Internalization by DCs of Anti-MR antibody B11 vs. internalization  
5                   by DCs of a mannosylated antigen (Inhibition of clathrin mediated  
                         internalization)**

Immature DCs can take up soluble antigens by pinocytic or receptor mediated endocytic mechanisms (55). The mechanism of antigen internalization  
10 determines its intracellular fate and may effect the quality of immune response to it (54, 55, 56). Internalization through the MR has been described as a rapid, clathrin mediated internalization event (57, 58). The MR itself has two putative clathrin targeting sequences within its cytoplasmic tail, and internalization of mannosylated gold particles have localized to clathrin-coated pits by EM (58, 59). Clathrin dependant endocytosis  
15 can be specifically disrupted by brief hypertonic shock or K<sup>+</sup> depletion (61). In order to determine if mannosylated antigens or B11 bound to the mannose receptor were internalized via clathrin-coated pits, immature DCs were incubated on ice in AIM5 media with or without 400mM sucrose for 30 min in the presence of either B11 mAb or mannosylated BSA. Cells were then warmed to 37°C and allowed to internalize for 20  
20 minutes. After being washed and fixed, cells were analyzed by confocal microscopy (data not shown). When B11 was bound to the MR, its uptake was inhibited by hypertonic shock, indicating that its mechanism of internalization was through clathrin coated-pits. Uptake of mannosylated BSA, in contrast, was not inhibited by hypertonic shock, indicating that its mechanism of internalization was not dependent on clathrin  
25 coated-pit formation. Even at concentration 20 fold higher than that of B11, surface staining by mannosylated BSA FITC was relatively weak. Subsequent studies revealed that internalized mannosylated BSA FITC co-localized with non-specific, fluid phase tracers, where as vesicles containing internalized B11 excluded the non-specific tracer (data not shown). In contrast to B11-FITC the uptake of both mannosylated BSA-FITC  
30 and the fluid phase tracer was largely blocked by pretreatment with the PI3K inhibitor wortmannin (data not shown). These results indicate that the vast majority of mannosylated BSA was taken up by the immature dendritic cell was through non-specific macropinocytic mechanisms, suggesting that the quality of immune response to the mannosylated antigen may differ greatly from antigen specifically targeted to the  
35 MR.

**Example 6 Binding of B11sfv- $\beta$ hCG to DCs**

Monocyte-derived DCs were exposed either to B11sfv- $\beta$ hCG or  $\beta$ hCG-B11 in PBS-BSA buffer for 45 minutes at 37°C and allowed to mature overnight in the presence of CD40L. Harvested DCs were then washed and stained with mouse anti- $\beta$ hCG followed by goat anti-hu IgG (F<sub>c</sub>)-PE conjugate. Stained cells were analyzed on a flow cytometer (BD-LSR). Approximately, 10,000 events were collected for each sample. Background autofluorescence and isotype matched antibody staining served as controls. Based on the mean fluorescence intensity (MFI) (data not shown), B11sfv- $\beta$ hCG binding to MR expressed on DC is similar to that of  $\beta$ hCG-B11.

**Example 7 CTLs specific for the  $\beta$ hCG-B11 construct recognize the scFv form of the antigen (B11sfv- $\beta$ hCG) presented by DCs**

CTL raised to DC-presented  $\beta$ hCG-B11 were tested against autologous DC targets that were exposed to  $\beta$ hCG-B11 and B11sfv- $\beta$ hCG, while untreated DC or DC exposed to B11 served as controls. Following antigen exposure, targets were labeled with <sup>51</sup>chromium and mixed with CTL in a 4 hour assay that measures release of radioactivity in the supernatant. In this experiment,  $\beta$ hCG-B11 -specific T cells recognize two of four targets that present the antigen on MHC class I molecules. No killing of targets ensues when DC lack antigen (Figure 11). Thus, the uptake of  $\beta$ hCG-B11 by DC likely results in a  $\beta$ hCG-derived T cell epitope recognized by CTL.

**Example 8 Production of transgenic mice which express the human mannose receptor**

The human mannose receptor (MR) targeting antibody does not cross-react with MR from other species commonly used for laboratory experiments, including monkeys. Therefore, transgenic mice were developed which express the human MR. Human MR-expressing transgenic mice were made using standard microinjection techniques using the entire 30 exon gene as well as additional sequences from 5' and 3' ends. The strain C57Bl/6 was chosen for the background of the human MR transgenic mice (huMR-tg) because immunization and tumor challenge experiments are well established for this strain. Using various methods, the expression and regulation of the human MR transgene was demonstrated to be similar to the expression and regulation of human MR in human tissues (data not shown).

**Example 9 Efficient loading of MR-expressing cells with B11- $\beta$ hCG**

The *in vivo* targeting capacity of B11- $\beta$ hCG was investigated by analyzing tissues from transgenic mice which express the human mannose receptor (hMR) 24 hours after administration of B11- $\beta$ hCG. Immunohistochemistry staining for the  $\beta$ hCG antigen in a lymph node and surrounding tissues near the injection site demonstrated a remarkable accumulation of the antigen in lymph node tissues and surrounding macrophages and interstitial dendritic cells (Figure 16). No staining was observed in control mice that do not express the hMR, showing that targeting hMR effectively loads antigens in antigen presenting cells and lymph nodes.

Specifically, B11- $\beta$ hCG (10  $\mu$ g) was injected into the limb of transgenic mice which express the human mannose receptor (huMR-tg mice) or WT littermates. After 24 hours, the nearby lymph nodes and surrounding tissue were removed and stained for  $\beta$ hCG. Tissue sections were stained with anti- $\beta$ hCG polyclonal antibody, and followed by a peroxidase labeled anti-rabbit IgG polymer reagent. As shown in Figure 16, no staining was observed in sections from WT mice, while huMR-tg mice demonstrated staining in lymph nodes and interstitial dendritic cells and macrophages of surrounding tissues.

**20 Example 10 Enhanced humoral and cellular responses to h $\beta$ CG when targeted to the MR**

The potential of antibody-targeted vaccines specific for MR were investigated by immunizing huMR-tg mice and their Wt littermates with B11- $\beta$ hCG. Antigen-specific humoral immunity to B11- $\beta$ hCG developed more rapidly and was greater in transgenic mice compared to non-transgenic littermates (see Figure 17). Specifically, as shown in Figure 17, mice were immunized 3 times (days 1, 15, and 29, as indicated with arrows) with 10  $\mu$ g of B11- $\beta$ hCG, or equivalent amount of free  $\beta$ hCG. The mice were bled one week after each immunization. Anti- $\beta$ hCG titers were determined by standard ELISA.

Similarly, huMR-tg mice immunized with B11- $\beta$ hCG exhibited a greater cellular immune response to B11- $\beta$ hCG compared to nontransgenic littermates (Figure 18). Specifically, as shown in Figure 18, Control (Wt) and huMR-tg mice were immunized with B11- $\beta$ hCG (1  $\mu$ g i.p. and adjuvant). Spleen cells were harvested 14 days later and analyzed for IFN- $\gamma$  production in response to  $\beta$ hCG peptides by standard ELISPOT assays. These studies further demonstrate the potency of targeting antigens to APCs *in vivo*. Moreover, humoral responses were readily observed in the absence of

adjuvants, cellular response to  $\beta$ hCG were more prominent when B11- $\beta$ hCG was co-administered with adjuvants such as poly-IC and CPG.

### Equivalents

- 5                    Those skilled in the art will recognize or be able to ascertain, using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

### 10    Incorporation by Reference

                  All patents, pending patent applications and other publications cited herein are hereby incorporated by reference in their entirety.

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We claim:

1. A molecular conjugate comprising a monoclonal antibody that binds to human antigen presenting cells (APCs) linked to  $\beta$  human chorionic gonadotropin ( $\beta$ hCG) and to an immunostimulatory agent.
2. The molecular conjugate of claim 1, wherein the  $\beta$ hCG and the immunostimulatory agent are linked to the antibody covalently, non-covalently, recombinantly or using a combination thereof.
3. The molecular conjugate of claim 1, wherein the  $\beta$ hCG and the immunostimulatory agent are both linked to the same chain of the antibody.
4. The molecular conjugate of claim 3, wherein the  $\beta$ hCG and the immunostimulatory agent are both linked to the heavy chain of the antibody.
5. The molecular conjugate of claim 3, wherein the  $\beta$ hCG and the immunostimulatory agent are both linked to the light chain of the antibody.
6. The molecular conjugate of claim 1, wherein the  $\beta$ hCG and the immunostimulatory agent are separately linked to the heavy and light chain of the antibody.
7. The molecular conjugate of claim 1, wherein the antibody, the  $\beta$ hCG and the immunostimulatory agent are expressed together as a single recombinant fusion protein.
8. The molecular conjugate of claim 1, wherein the immunostimulatory agent is selected from the group consisting of a CD40 ligand, a cytokine, a colony-stimulating factor, an anti-CTLA-4 antibody, a toll receptor agonist, LPS (endotoxin), R837, R848, polyI:C (inosine:cytosine polynucleotide), ssRNA, dsRNA, Bacille Calmette-Guerin (BCG), Levamisole hydrochloride, and an intravenous immune globulin.
9. The molecular conjugate of claim 8, wherein the cytokine is selected from the group consisting of IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$  and IL-2.

10. The molecular conjugate of claim 8, wherein the toll receptor agonist is flagellin or MALP-2.
11. The molecular conjugate of claim 1, wherein the antibody binds to  
5 a C-type lectin expressed on human dendritic cells.
12. The molecular conjugate of claim 1, wherein the antibody binds to the human mannose receptor.
13. The molecular conjugate of claim 1, wherein the antibody is  
10 selected from the group consisting of human, humanized and chimeric antibodies.
14. The molecular conjugate of claim 1, wherein the antibody is selected from the group consisting of a whole antibody, an Fab fragment and a single  
15 chain antibody.
15. The molecular conjugate of claim 1, wherein the conjugate is a recombinant fusion protein.
16. The molecular conjugate of claim 1, wherein the antibody  
20 comprises a human heavy chain variable region comprising FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4 sequences and a human light chain variable region comprising FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4 sequences, wherein:
- (a) the human heavy chain variable region CDR3 sequence comprises  
25 SEQ ID NO: 15, and conservative modifications thereof; and
- (b) the human light chain variable region CDR3 sequence comprises SEQ ID NO: 18, and conservative modifications thereof.
17. The molecular conjugate of claim 16, wherein the human heavy  
30 chain variable region CDR2 sequence comprises SEQ ID NO: 14, and conservative modifications thereof; and the human light chain variable region CDR2 sequence comprises SEQ ID NO:17, and conservative modifications thereof.
18. The molecular conjugate of claim 16, wherein the human heavy  
35 chain variable region CDR1 sequence comprises SEQ ID NO:13, and conservative modifications thereof; and the human light chain variable region CDR1 sequence comprises SEQ ID NO:16, and conservative modifications thereof.



19. The molecular conjugate of claim 1, wherein the antibody comprises:
- (a) a heavy chain variable region derived from a human VH5-51 germline sequence (SEQ ID NO:30); and
  - 5 (b) a light chain variable region derived from a human Vk-L15 (SEQ ID NO:32) germline sequence.
20. The molecular conjugate of claim 1, wherein the antibody comprises human heavy chain and human light chain variable regions comprising the
- 10 amino acid sequences shown in SEQ ID NO:4 and SEQ ID NO:8, respectively, or an amino acid sequence that is sufficiently homologous to SEQ ID NO:4 or SEQ ID NO:8 such that the antibody retains the ability to bind to human dendritic cells.
21. A molecular conjugate comprising a human antibody heavy chain
- 15 that binds to human APCs and a human antibody light chain that binds to human APCs, wherein either or both chains are linked to  $\beta$ hCG and to an immunostimulatory agent.
22. A molecular conjugate comprising a human antibody heavy chain that binds to human APCs and a human antibody light chain that binds to human APCs,
- 20 wherein one of the chains is linked to  $\beta$ hCG and the other chain is linked to an immunostimulatory agent.
23. The molecular conjugate of claim 21, wherein the heavy chain is linked to  $\beta$ hCG and comprises the amino acid sequence shown in SEQ ID NO:2.
- 25
24. The molecular conjugate of claim 21, wherein the light chain comprises the amino acid sequence shown in SEQ ID NO:6.
25. A molecular conjugate comprising:
- 30 a monoclonal antibody that binds to human antigen presenting cells (APCs);
- $\beta$  human chorionic gonadotropin ( $\beta$ hCG); and
- an immunostimulatory agent, wherein the antibody comprises:
- (a) a heavy chain variable region derived from a human VH5-51
  - 35 germline sequence (SEQ ID NO:30); and
  - (b) a light chain variable region derived from a human Vk-L15 (SEQ ID NO:32) germline sequence.

26. A molecular conjugate comprising the amino acid sequence shown in SEQ ID NO:12 linked to an immunostimulatory agent.
27. The molecular conjugate of claim 1 which is internalized and processed by APCs, such that a T cell-mediated immune response is generated against the antigen.
28. The molecular conjugate of claim 27, wherein the T cell response is mediated by cytotoxic T cells.
29. The molecular conjugate of claim 27, wherein the T cell response is mediated by both CD4<sup>+</sup> and CD8<sup>+</sup> T cells.
30. The molecular conjugate of claim 27, wherein the T cell response is induced through both MHC class I and MHC class II pathways.
31. A composition comprising an immunostimulatory agent and a molecular conjugate of an antibody that binds to APCs linked to  $\beta$ hCG.
32. The composition of claim 31, wherein the immunostimulatory agent is linked to the molecular conjugate.
33. The composition of claim 32, wherein the linkage is covalent, non-covalent or recombinant.
34. The composition of claim 31, wherein the antibody is a human antibody.
35. The composition of claim 31, further comprising a pharmaceutically acceptable carrier.
36. The composition of claim 31, further comprising an adjuvant.
37. The composition of claim 31, wherein the immunostimulatory agent is selected from the group consisting of a CD40 ligand, a cytokine, a colony-stimulating factor, an anti-CTLA-4 antibody, a toll receptor agonist, LPS (endotoxin), R837, R848, polyI:C (inosine:cytosine polynucleotide), ssRNA, dsRNA, Bacille

Calmette-Guerin (BCG), Levamisole hydrochloride, and an intravenous immune globulin.

38. The composition of claim 37, wherein the cytokine is selected  
5 from the group consisting of IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$  and IL-2.

39. A method of inducing or enhancing a T cell-mediated immune  
response against  $\beta$ hCG, comprising contacting the composition of claim 31 with APCs  
such that the antigen is processed and presented to T cells in a manner which induces or  
10 enhances a T cell-mediated response against the antigen.

40. The method of claim 39, wherein the immunostimulatory agent is  
linked to the molecular conjugate.

41. A method of immunizing a subject comprising administering a  
15 composition of claim 31 such that a T cell-mediated immune response is generated  
against  $\beta$ hCG.

42. The method of claim 41, wherein the immunostimulatory agent is  
20 linked to the molecular conjugate.

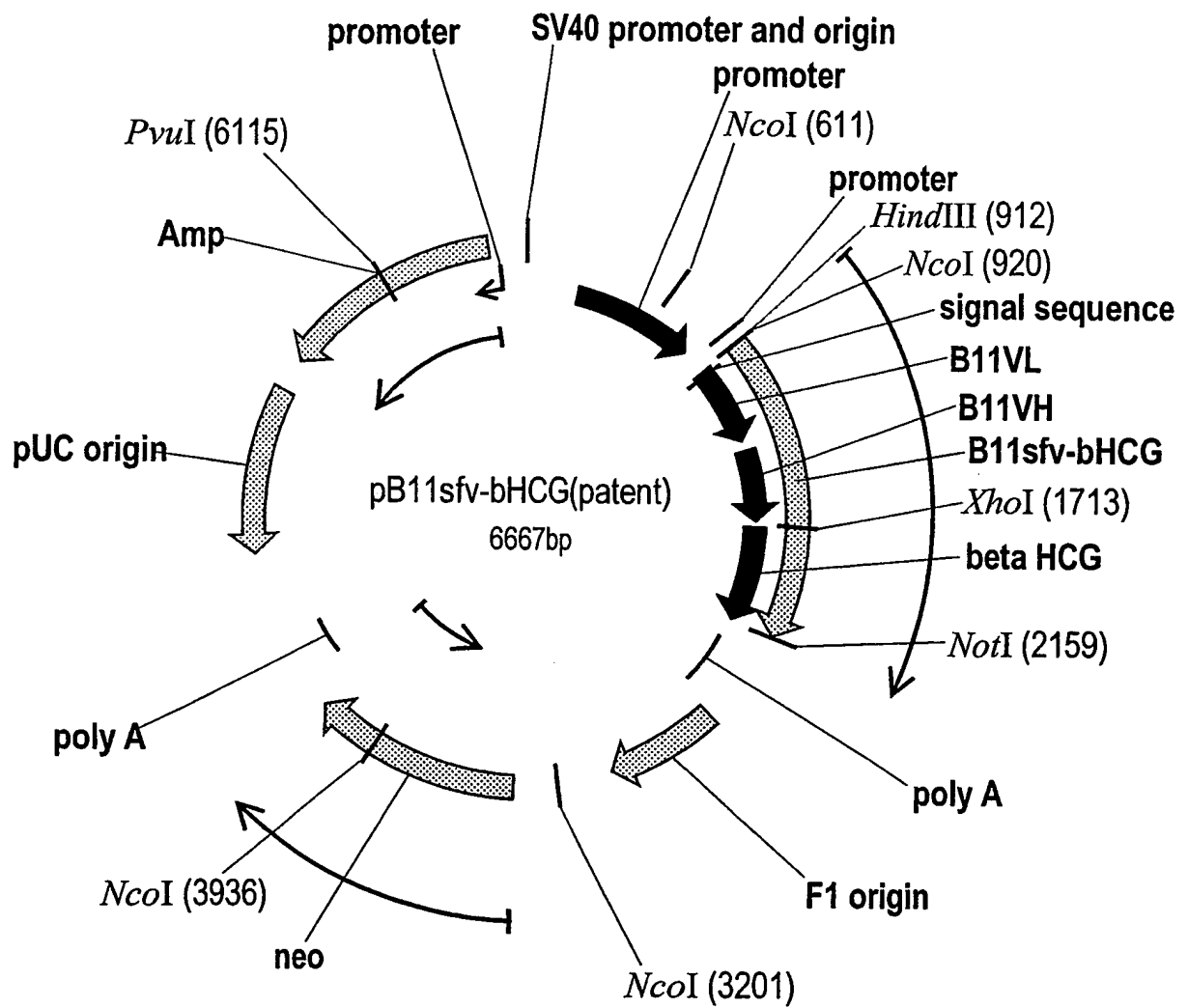
43. The method of claim 41, further comprising the step of  
administering an adjuvant.

44. The method of claim 41, wherein the immunostimulatory agent is  
25 selected from the group consisting of a CD40 ligand, a cytokine, a colony-stimulating  
factor, an anti-CTLA-4 antibody, a toll receptor agonist, LPS (endotoxin), R837, R848,  
polyI:C (inosine:cytosine polynucleotide), ssRNA, dsRNA, Bacille Calmette-Guerin  
(BCG), Levamisole hydrochloride, and an intravenous immune globulin.

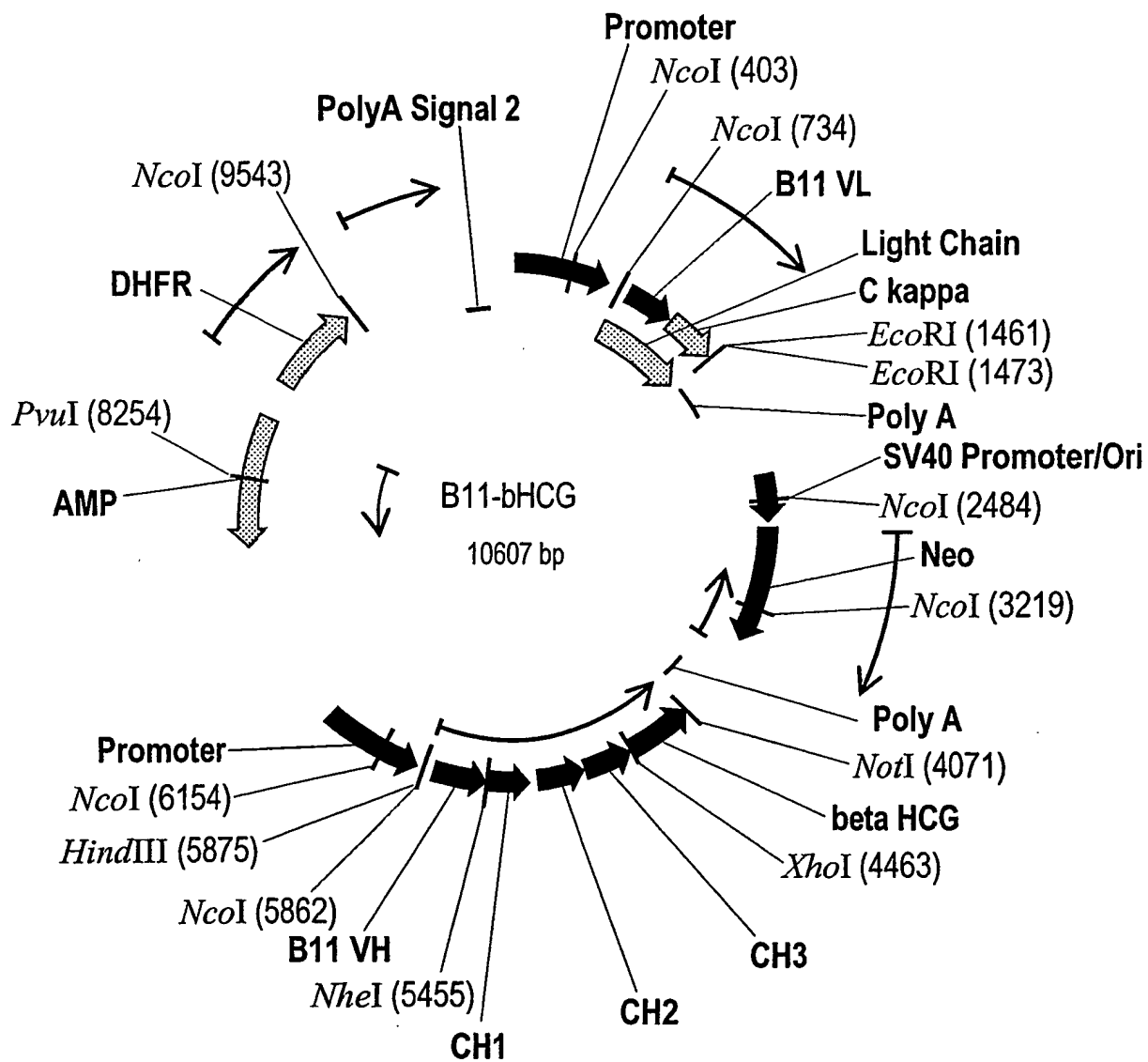
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45. The method of claim 44, wherein the cytokine is selected from the  
group consisting of IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$  and IL-2.

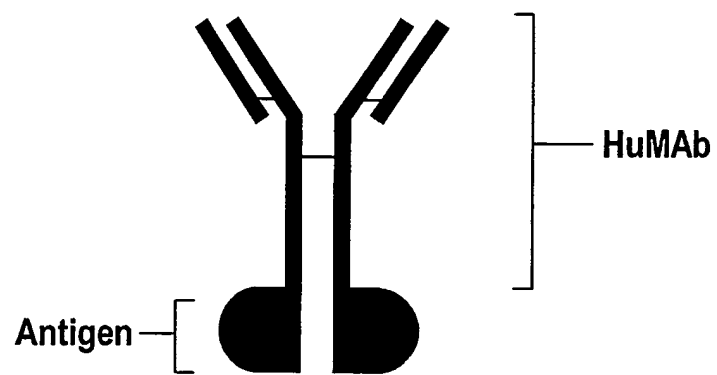
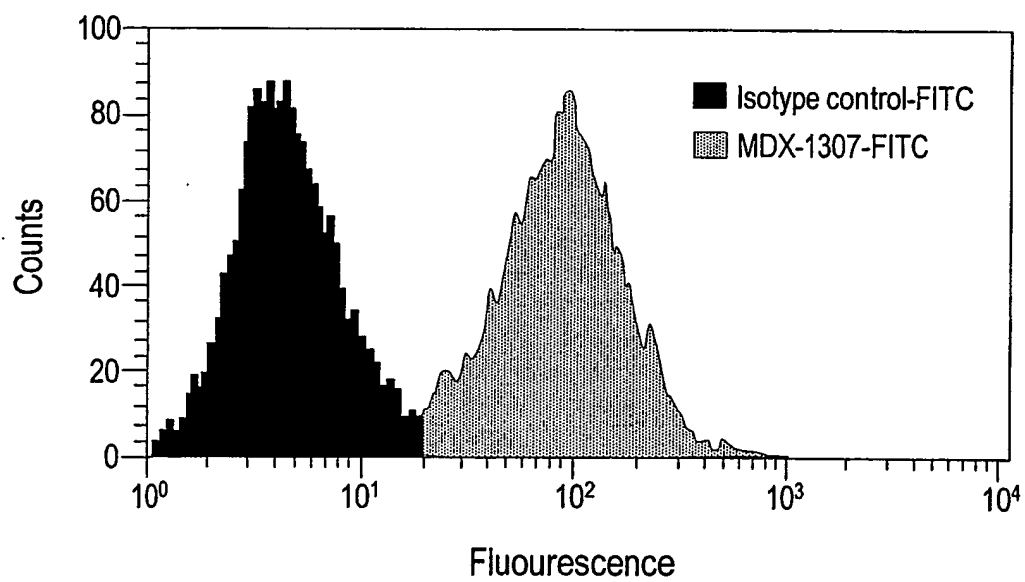
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*Fig. 1*

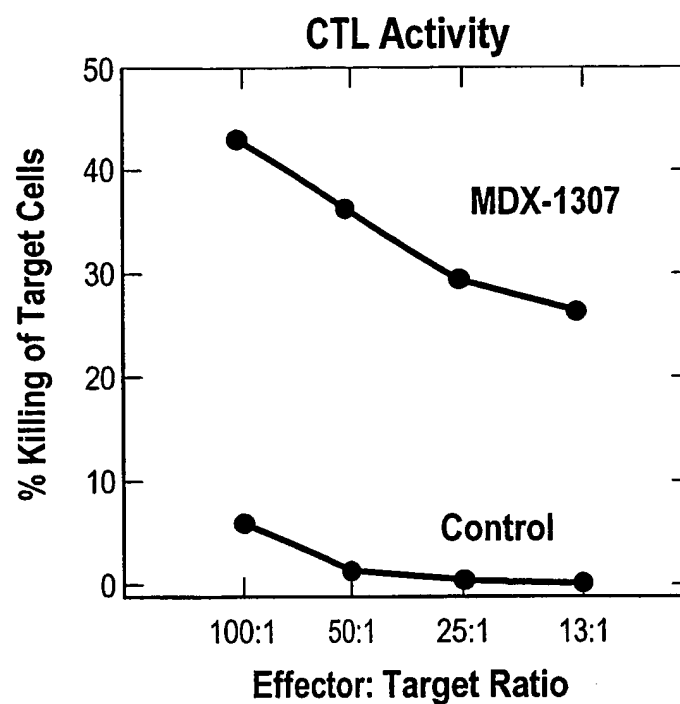
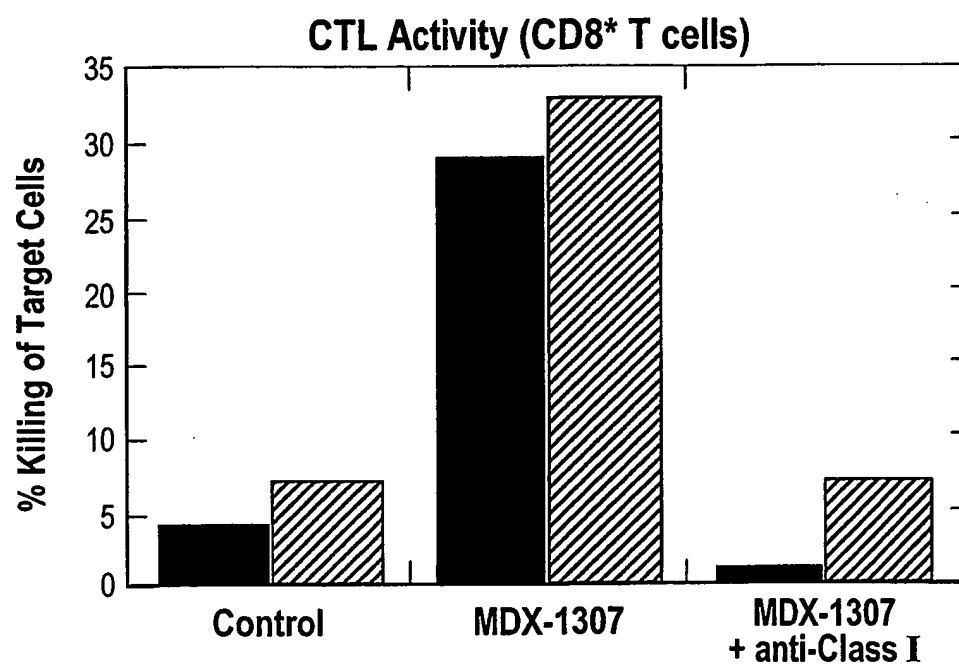
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*Fig. 2*

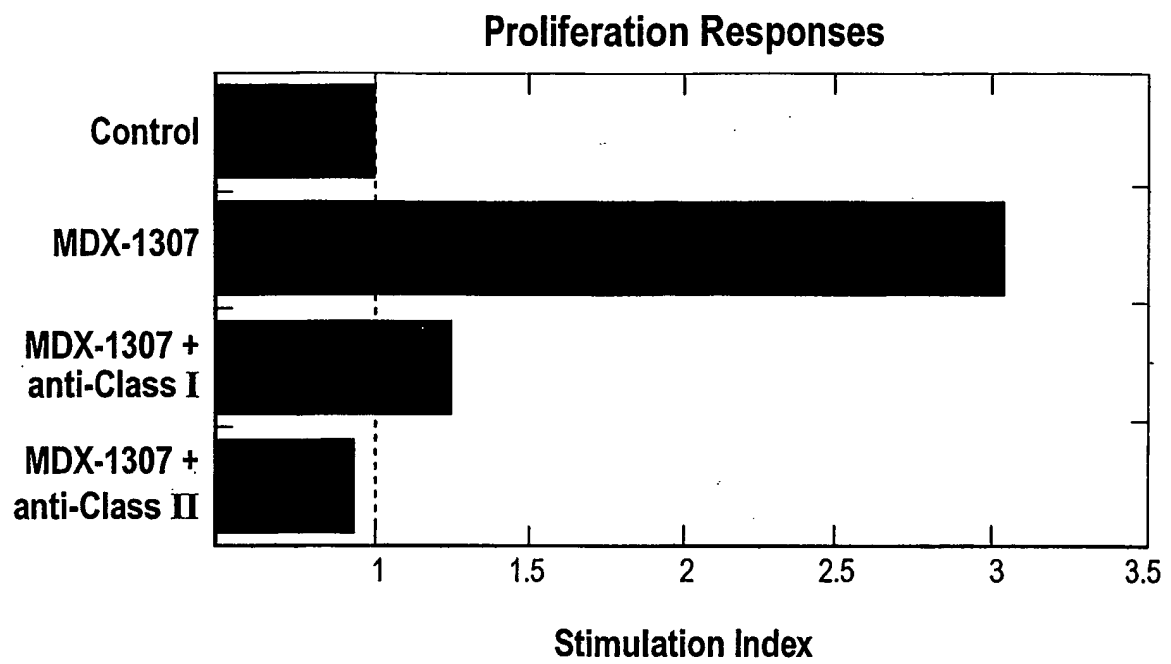
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*Fig. 3**Fig. 4*

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*Fig. 5**Fig. 6*

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*Fig. 7*



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## Anti-Mannose Receptor B11 VH

V-segment: Locus - 5-51 Name: DP-73/V5-51

D-segment: Unknown

J-segment: JH4b

```

      E  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  E  S  L
1   GAG GTG CAG CTG GTG CAG TCT GGA GCA GAG GTG AAA AAG CCC GGG GAG TCT CTG

                                     CDR 1
                                     ~~~~~
      R  I  S  C  K  G  S  G  D  S  F  T  T  Y  W  I  G  W
55  AGG ATC TCC TGT AAG GGT TCT GGA GAC AGT TTT ACC ACC TAC TGG ATC GGC TGG

                                     CDR 2
                                     ~~~~~
      V  R  Q  M  P  G  K  G  L  E  W  M  G  I  I  Y  P  G
109 GTG CGC CAG ATG CCC GGG AAA GGC CTG GAG TGG ATG GGG ATC ATC TAT CCT GGT

      CDR 2
      ~~~~~
      D  S  D  T  I  Y  S  P  S  F  Q  G  Q  V  T  I  S  A
163 GAC TCT GAT ACC ATA TAC AGC CCG TCC TTC CAA GGC CAG GTC ACC ATC TCA GCC

      D  K  S  I  S  T  A  Y  L  Q  W  S  S  L  K  A  S  D
217 GAC AAG TCC ATC AGC ACC GCC TAC CTG CAG TGG AGC AGC CTG AAG GCC TCG GAC

      CDR 3
      ~~~~~
      T  A  M  Y  Y  C  T  R  G  D  R  G  V  D  Y  W  G  Q
271 ACC GCC ATG TAT TAC TGT ACG AGA GGG GAC CGG GGC GTT GAC TAC TGG GGC CAG
                                     |
                                     L JH4b

      G  T  L  V  T  V  S  S
325 GGA ACC CTG GTC ACC GTC TCC TCA

```

*Fig. 8*

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## Anti-Mannose Receptor B11 VL

V-segment: Locus - L15

Name: DPK7/HK134

J-segment: JK1

```
      D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R
1    GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT GCA TCT GTA GGA GAC AGA

                                CDR 1
                                -----
      V   T   I   T   C   R   A   S   Q   G   I   S   R   W   L   A   W   Y
55    GTC ACC ATC ACT TGT CGG GCG AGT CAG GGT ATT AGC AGG TGG TTA GCC TGG TAT

                                CDR 2
                                -----
      Q   Q   K   P   E   K   A   P   K   S   L   I   Y   A   A   S   S   L
109   CAG CAG AAA CCA GAG AAA GCC CCT AAG TCC CTG ATC TAT GCT GCA TCC AGT TTG

      CDR 2
      -----
      Q   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163   CAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

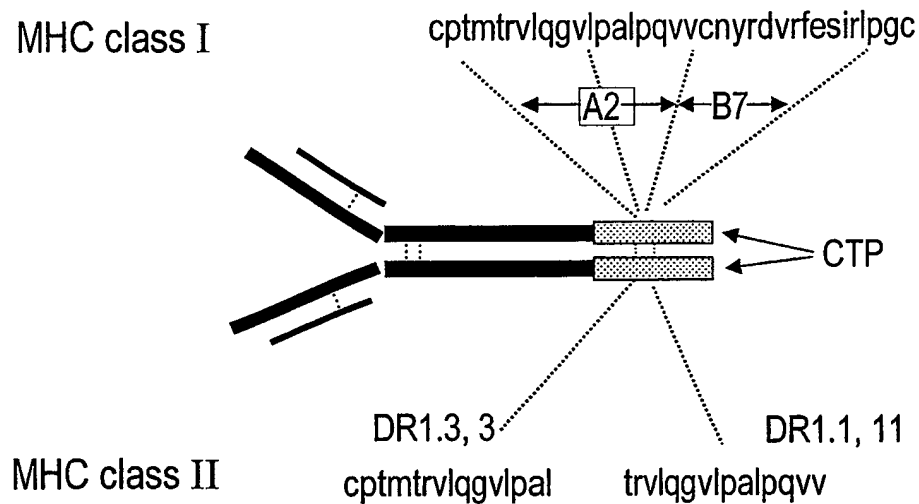
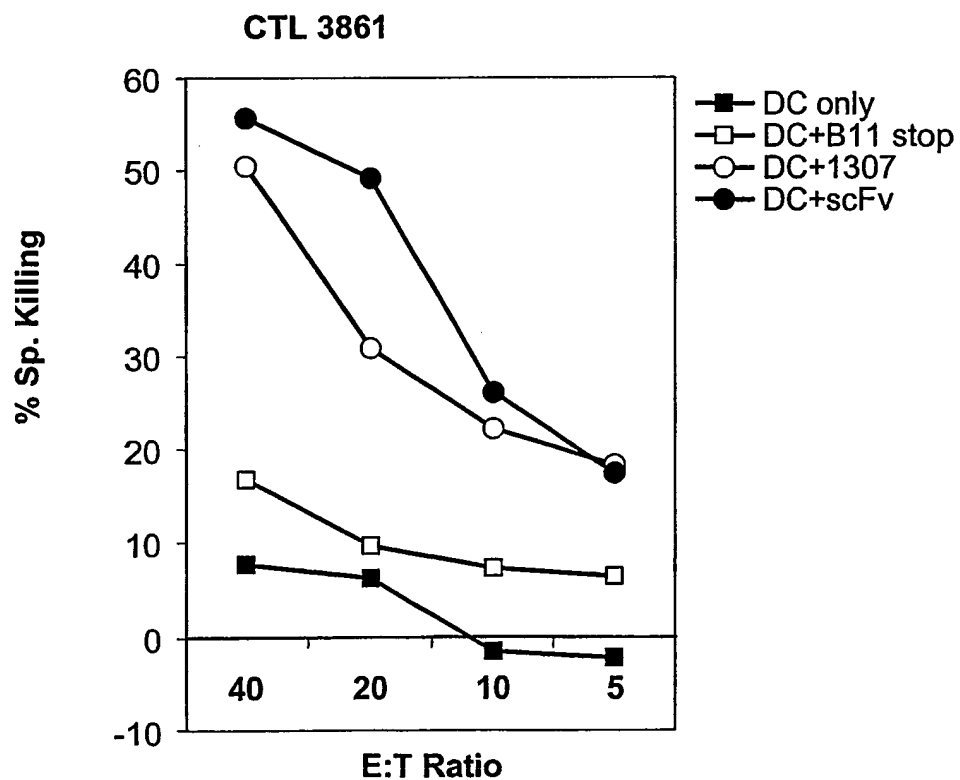
                                CDR 3
                                -----
      L   T   I   S   G   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217   CTC ACC ATC AGC GGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGC CAA CAG

      CDR 3
      -----
      Y   N   S   Y   P   R   T   F   G   Q   G   T   K   V   E   I   K
271   TAT AAT AGT TAC CCT CGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA

                                JK 1
                                ↓
```

*Fig. 9*

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*Fig. 10**Fig. 11*

## Anti-Mannose Receptor VH5-51 Regions

	CDR1																																		
5-51 germline	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	E	S	L	K	I	S	C	K	G	S	G	Y	S	F	T	S	Y	W	I	G
B11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	-	-	-	-	-	-	-	D	-	-	-	-	T	-	-	-

[illegible]

	5-51 germline	CDR3
B11	S A D K S I S T A Y L Q W S S L K A S D T A M Y C A R	
	- - - - -	- - - - - T - G D R G V D Y

5-51 germline  
B11  
W G Q G T L V T V S S

Fig. 12

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## Anti-Mannose Receptor VH regions (VH5-51):

VH5-51

**Germline:** GAG GTG CAG CTG GTG CAG TCT GGA GCA GAG GTG AAA AAG CCC GGG GAG TCT CTG AAG ATC TCC TGT AAG GGT  
**B11:** --- --- --- --- --- --- --- --- -G- --- ---

CDRI

**v germline:** TCT GGA TAC AGC TTT ACC AGC TAC TGG ATC GGC TGG GTG CGC CAG ATG CCC GGG AAA GGC CTG GAG TGG ATG  
**B11:** --- --- G--- --T --- -C- --- --- --- --- --- --- ---

CDR2

```

v germline: GGG ATC ATC TAT CCT GGT GAC TCT GAT ACC AGA TAC AGC CCG TCC TTC CAA GGC CAG GTC ACC ATC TCA GCC
B11:
-----
-T-----

```

CDR3

V germline: GCG AGA  
B11: A--- --- GGG GAC CGG GGC GGC GTT GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA

Fig. 13

## Anti-Mannose Receptor V $\alpha$ L15 Regions

	L15 germline	Q K P E K A P K S L I Y A A S S L Q S G V P S R F S G S G S G T D F T L T	
	B11	- -	
		<u>CDR2</u>	

Fig. 14

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## Anti-Mannose Receptor VK Regions

L15 germline: GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC ATC  
 B11: ---

CDR1  
 L15 germline: ACT TGT CGG GCG AGT CAG GGT ATT AGC AGC TGG TTA GCC TGG TAT CAG CAG AAA CCA GAG AAA  
 B11: ---

CDR2  
 L15 germline: GCC CCT AAG TCC CTG ATC TAT GCT GCA TCC AGT TTG CAA AGT GGG GTC CCA TCA AGG TTC AGC  
 B11: ---

L15 germline: GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGC CTG GAG CCT GAA GAT TTT GCA  
 B11: ---

CDR3  
 L15 germline: ACT TAT TAC TGC CAA CAG TAT AAT AGT TAC CCT  
 B11: ---

B11: ATC AAA

Fig. 15

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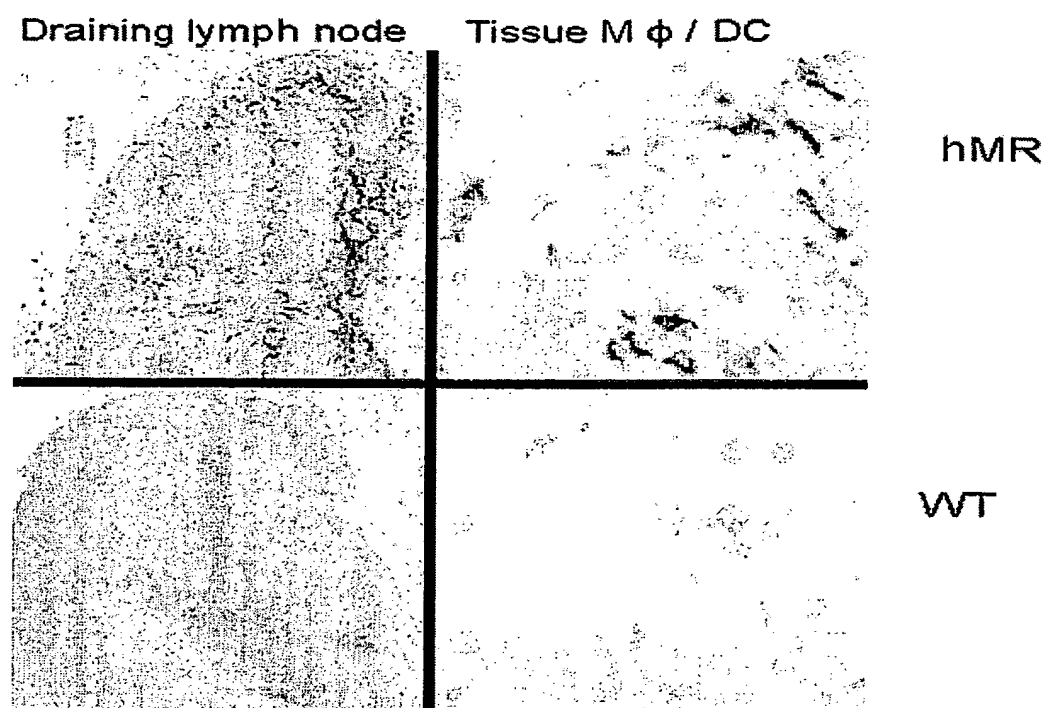


Figure 16



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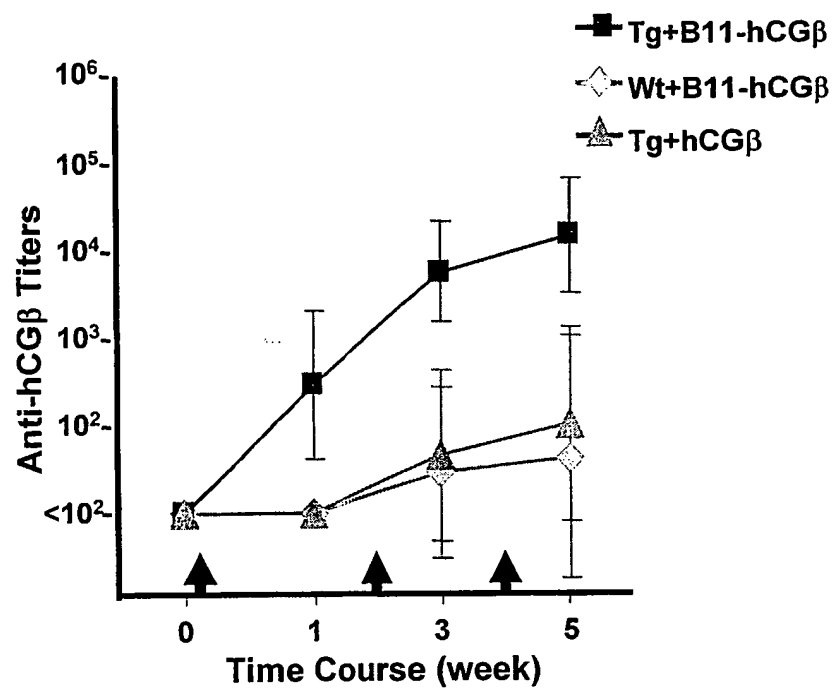
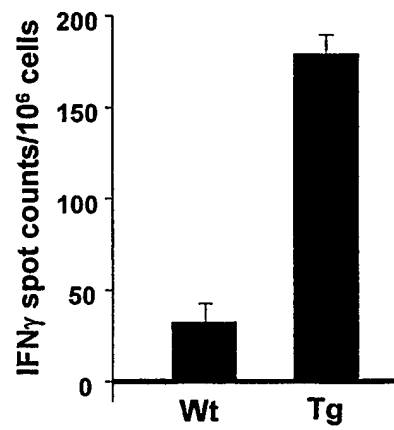


Figure 17

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**hCG $\beta$ -specific T cell response****Figure 18**

## SEQUENCE LISTING

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<120> ANTIBODY VACCINE CONJUGATES AND USES  
THEREFOR

&lt;130&gt; MXI-301CPPC

&lt;150&gt; 10/903191

&lt;151&gt; 2004-07-30

&lt;150&gt; 10/769144

&lt;151&gt; 2004-01-30

&lt;150&gt; 60/443979

&lt;151&gt; 2003-01-31

&lt;160&gt; 32

&lt;170&gt; FastSEQ for Windows Version 4.0

&lt;210&gt; 1

&lt;211&gt; 1407

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 1

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&lt;210&gt; 2

&lt;211&gt; 468

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 2

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Val His Ser Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
      20             25             30
Pro Gly Glu Ser Leu Arg Ile Ser Cys Lys Gly Ser Gly Asp Ser Phe
      35             40             45

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Thr	Thr	Tyr	Trp	Ile	Gly	Trp	Val	Arg	Gln	Met	Pro	Gly	Lys	Gly	Leu
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Glu	Trp	Met	Gly	Ile	Ile	Tyr	Pro	Gly	Asp	Ser	Asp	Thr	Ile	Tyr	Ser
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Pro	Ser	Phe	Gln	Gly	Gln	Val	Thr	Ile	Ser	Ala	Asp	Lys	Ser	Ile	Ser
			85						90					95	
Thr	Ala	Tyr	Leu	Gln	Trp	Ser	Ser	Leu	Lys	Ala	Ser	Asp	Thr	Ala	Met
			100					105					110		
Tyr	Tyr	Cys	Thr	Arg	Gly	Asp	Arg	Gly	Val	Asp	Tyr	Trp	Gly	Gln	Gly
		115				120						125			
Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe
	130					135					140				
Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu
145					150				155						160
Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp
			165						170					175	
Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu
			180					185					190		
Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser
		195					200					205			
Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro
		210				215					220				
Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys
225					230				235						240
Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro
			245						250					255	
Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser
		260						265					270		
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp
		275					280						285		
Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn
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Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val
305					310					315					320
Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu
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Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys
			340					345					350		
Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr
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Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr
		370				375					380				
Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu
385					390					395					400
Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu
			405						410					415	
Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys
			420					425					430		
Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu
		435				440						445			
Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly
	450					455						460			
Lys	Gly	Ser	Ser												
465															

&lt;210&gt; 3

&lt;211&gt; 348

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 3

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 cccgggaaag gcctggagtg gatggggatc atctatcctg gtgactctga taccatatac 180

agccccgtcct tccaaggcca gggtcaccatc tcagccgaca agtccatcag caccgcctac 240  
 ctgcagtggg gcagcctgaa ggcctcggac accgccatgt attactgtac gagaggggac 300  
 cggggcggtg actactgggg ccagggaacc ctggtcaccg tctcctca 348

<210> 4  
 <211> 116  
 <212> PRT  
 <213> Homo sapiens

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 20 25 30  
 Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met  
 35 40 45  
 Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Ile Tyr Ser Pro Ser Phe  
 50 55 60  
 Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr  
 65 70 75 80  
 Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys  
 85 90 95  
 Thr Arg Gly Asp Arg Gly Val Asp Tyr Trp Gly Gln Gly Thr Leu Val  
 100 105 110  
 Thr Val Ser Ser  
 115

<210> 5  
 <211> 702  
 <212> DNA  
 <213> Homo sapiens

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 acttgctcggg cgagtcaggg tattagcagg tggtagcct ggtatcagca gaaaccagag 180  
 aaagccccta agtccctgat ctatgctgca tccagtttgc aaagtggggc cccatcaagg 240  
 ttcagcggca gtggatctgg gacagatttc actctcacca tcagcggcct gcagcctgaa 300  
 gattttgcaa cttattactg ccaacagtat aatagttacc ctcggacgtt cggccaaggg 360  
 accaagggtg aaatcaaacg tacggtggcg gcgccatctg tcttcatctt cccgccatct 420  
 gatgagcagt tgaaatctgg aactgcctct gttgtgtgcc tgctgaataa cttctatccc 480  
 agagaggcca aagtacagtg gaagggtggat aacgccctcc aatcgggtaa ctcccaggag 540  
 agtgtcacag agcaggacag caaggacagc acctacagcc tcagcagcac cctgacgctg 600  
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<210> 6  
 <211> 233  
 <212> PRT  
 <213> Homo sapiens

<400> 6  
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 Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile  
 35 40 45  
 Ser Arg Trp Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys  
 50 55 60  
 Ser Leu Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg  
 65 70 75 80  
 Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Gly  
 85 90 95

Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser  
 100 105 110  
 Tyr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr  
 115 120 125  
 Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu  
 130 135 140  
 Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro  
 145 150 155 160  
 Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly  
 165 170 175  
 Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr  
 180 185 190  
 Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His  
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 210 215 220  
 Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 225 230

<210> 7  
 <211> 321  
 <212> DNA  
 <213> Homo sapiens

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 gagaaagccc ctaagtcctt gatctatgct gcatccagtt tgcaaagtgg ggtcccatca 180  
 aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcgg cctgcagcct 240  
 gaagattttg caacttatta ctgccaacag tataatagtt accctcggac gttcggccaa 300  
 gggaccaagg tggaaatcaa a 321

<210> 8  
 <211> 107  
 <212> PRT  
 <213> Homo sapiens

<400> 8  
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 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Arg Trp  
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 Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile  
 35 40 45  
 Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60  
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Gly Leu Gln Pro  
 65 70 75 80  
 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro Arg  
 85 90 95  
 Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> 9  
 <211> 1842  
 <212> DNA  
 <213> Homo sapiens

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 tgtaagggtt ctggagacag ttttaccacc tactggatcg gctgggtgcg ccagatgccc 180  
 gggaaaggcc tggagtggat ggggatcatc tatcctggtg actctgatac catatacagc 240

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cgactcccg ggcctcggga cccccgatc ctcccacaat aa 1842

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&lt;210&gt; 10

&lt;211&gt; 613

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 10

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Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
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Val His Ser Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
 20          25          30
Pro Gly Glu Ser Leu Arg Ile Ser Cys Lys Gly Ser Gly Asp Ser Phe
 35          40          45
Thr Thr Tyr Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu
 50          55          60

Glu Trp Met Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Ile Tyr Ser
 65          70          75          80
Pro Ser Phe Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser
 85          90          95
Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met
100          105          110
Tyr Tyr Cys Thr Arg Gly Asp Arg Gly Val Asp Tyr Trp Gly Gln Gly
115          120          125
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
130          135          140
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
145          150          155          160
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165          170          175
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180          185          190
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
195          200          205
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
210          215          220
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
225          230          235          240

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Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro  
 245 250 255  
 Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser  
 260 265 270  
 Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp  
 275 280 285  
 Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn  
 290 295 300  
 Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val  
 305 310 315 320  
 Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu  
 325 330 335  
 Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys  
 340 345 350  
 Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr  
 355 360 365  
 Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr  
 370 375 380  
 Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu  
 385 390 395 400  
 Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu  
 405 410 415  
 Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys  
 420 425 430  
 Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu  
 435 440 445  
 Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
 450 455 460  
 Lys Gly Ser Ser Ser Lys Glu Pro Leu Arg Pro Arg Cys Arg Pro Ile  
 465 470 475 480  
 Asn Ala Thr Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr  
 485 490 495  
 Val Asn Thr Thr Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val  
 500 505 510  
 Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg  
 515 520 525  
 Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val  
 530 535 540  
  
 Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu  
 545 550 555 560  
 Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu  
 565 570 575  
 Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Lys Ala Pro  
 580 585 590  
 Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr  
 595 600 605  
 Pro Ile Leu Pro Gln  
 610

&lt;210&gt; 11

&lt;211&gt; 1325

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 11

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 agtcaccatc acttgctcgg cgagtcaggg tattagcagg tggttagcct ggtatcagca 180  
 gaaaccagag aaagccccta agtccctgat ctatgctgca tccagtttgc aaagtgggggt 240  
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 gtctctgagg atctcctgta agggttctgg agacagtttt accacctact ggatcggctg 540



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&lt;210&gt; 12

&lt;211&gt; 411

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 12

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Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
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Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala
          20          25          30
Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile
          35          40          45
Ser Arg Trp Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys
          50          55          60
Ser Leu Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg
65          70          75          80
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Gly
          85          90          95

Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser
          100          105          110
Tyr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly Gly
          115          120          125
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln
          130          135          140
Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser Leu Arg
145          150          155          160
Ile Ser Cys Lys Gly Ser Gly Asp Ser Phe Thr Thr Tyr Trp Ile Gly
          165          170          175
Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly Ile Ile
          180          185          190
Tyr Pro Gly Asp Ser Asp Thr Ile Tyr Ser Pro Ser Phe Gln Gly Gln
          195          200          205
Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu Gln Trp
          210          215          220
Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Thr Arg Gly
225          230          235          240
Asp Arg Gly Val Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
          245          250          255
Ser Gly Ser Thr Gly Gly Gly Gly Ser Ser Lys Glu Pro Leu Arg
          260          265          270
Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu Gly
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Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala Gly Tyr Cys
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Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
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 50 55 60  
 Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val  
 65 70 75 80  
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 85 90 95  
 Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp  
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&lt;212&gt; PRT

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 Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
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 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro  
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(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
13 July 2006 (13.07.2006)

PCT

(10) International Publication Number  
**WO 2006/073493 A3**

(51) International Patent Classification:

A61K 47/48 (2006.01) A61P 37/04 (2006.01)  
A61K 39/395 (2006.01)

(21) International Application Number:

PCT/US2005/027044

(22) International Filing Date: 28 July 2005 (28.07.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

10/903,191 30 July 2004 (30.07.2004) US

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(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AL, AM,  
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,  
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,  
KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,  
MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ,  
OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL,  
SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC,  
VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): ARIPO (BW, GH,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,  
RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,  
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(88) Date of publication of the international search report:  
4 January 2007

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: VACCINE CONJUGATES COMPRISING A MONOCLONAL ANTIBODY BINDING TO HUMAN DENDRITIC  
CELLS AND BETA HUMAN CHRONIC GONADOTROPIN

(57) Abstract: The present invention provides novel antibody vaccine conjugates and methods of using the same to induce a cy-  
totoxic T cell (CTL) response. In a particular, embodiment, the vaccine conjugate includes a human chorionic gonadotropin beta  
subunit ( $\beta$ hCG) antigen linked to an anti-mannose receptor (MR) antibody.

WO 2006/073493 A3

PCT/US2005/027044

## A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K47/48 A61K39/395 A61P37/04

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBASE, BIOSIS, WPI Data, PAJ, Sequence Search

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>HE L-Z ET AL: "A Novel Human Cancer Vaccine Elicits Cellular Responses to the Tumor-Associated Antigen, Human Chorionic Gonadotropin [beta]"</p> <p>CLINICAL CANCER RESEARCH 15 MAR 2004 UNITED STATES, vol. 10, no. 6, 15 March 2004 (2004-03-15), pages 1920-1927, XP002393832 ISSN: 1078-0432 the whole document</p> <p style="text-align: center;">----- -/--</p>	1-45

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

## \* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

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\*G\* document member of the same patent family

Date of the actual completion of the international search

11 August 2006

Date of mailing of the international search report

29/08/2006

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Le Flao, K

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>HE LIZHEN ET AL: "An antigen presenting cell-targeted cancer vaccine that elicits CD4 and CD8 effector responses to the hCGbeta tumor-associated antigen."            PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 44, July 2003 (2003-07), page 167, XP001208026            &amp; 94TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH; WASHINGTON, DC, USA; JULY 11-14, 2003            ISSN: 0197-016X            abstract</p>	1-45
A	<p>RAMAKRISHNA V ET AL: "Mannose receptor targeting of tumor antigen pmel17 to human dendritic cells directs anti-melanoma T cell responses via multiple HLA molecules"            JOURNAL OF IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIMORE, US, vol. 172, no. 5, 1 March 2004 (2004-03-01), pages 2845-2852, XP002376446            ISSN: 0022-1767            abstract</p>	1-45
A	<p>WO 03/040169 A (MEDAREX, INC; DEO, YASHWANT, M; KELER, TIBOR)            15 May 2003 (2003-05-15)            examples 1,2</p>	1-45
A	<p>FRLETA D ET AL: "Class II-targeted antigen is superior to CD40-targeted antigen at stimulating humoral responses in vivo."            INTERNATIONAL IMMUNOPHARMACOLOGY. FEB 2001, vol. 1, no. 2, February 2001 (2001-02), pages 265-275, XP002394474            ISSN: 1567-5769            the whole document</p>	1-45
P,X	<p>RAMAKRISHNA VENKY ET AL: "Synergistic role of TLR Agonists in T cell-mediated immunity induced by mannose receptor antibody targeting of tumor antigens to human DCs"            JOURNAL OF IMMUNOTHERAPY, vol. 28, no. 6, November 2005 (2005-11), page 658, XP008067471            &amp; 20TH ANNUAL SCIENTIFIC MEETING OF THE INTERNATIONAL-SOCIETY-FOR-BIOLOGICAL-THERAPY-OF-CANCER; ALEXANDRIA, VA, USA; NOVEMBER 10 -13, 2005            ISSN: 1524-9557            abstract</p>	1-45



**Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 41-\*45 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03040169	A	15-05-2003	CA 2466049 A1 15-05-2003
			CN 1612934 A 04-05-2005
			EP 1448787 A2 25-08-2004
			JP 2006501131 T 12-01-2006
			MX PA04004324 A 16-05-2005

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